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"Everybody comes to a point in their life when they want to quit.

But it's what you do at that moment that determines who you are."

David Goggins

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Abbreviations

AACC : American Association of Cereal Chemists.

AM : Torque 15 s before the PMT in the GPT curve.

AU : Arbitrary units.

BE : Brabender equivalents.

BEV : Blending effect value.

CEN : European Committee for Standardization

DM : Dry matter.

DTT : Dithiothreitol.

E : Dough extensibility.

En : Energy.

GMP : Glutenin macropolymer.

GPT : Gluto-Peak®-Test.

HMW : High molecular weight.

HMW-GS : High molecular weight-glutenin subunits.

ICC : International Association for Cereal Science and Technology.

LMW : Low molecular weight.

LMW-GS : Low molecular weight-glutenin subunits.

LV : Loaf volume.

MT : Maximum torque in the GPT curve.

NITs : Near Infrared Transmission spectroscopy.

PC : Protein content.

Ph Eur : European Pharmacopoeia

PM : Torque 15 s after the PMT in the GPT curve.

PMT : Peak maximum time in the GPT curve.

r : Coefficient of correlation.

R : Resistance to extension.

RMT **:** Rapid Mix Test.

RSQ **:** Coefficient of determination (R2).

S.E **:** Standard Error of Estimate.

SDS-PAGE : Sodium dodecyl sulphate polyacrylamide gel electrophoresis.

SRC **:** Solvent Retention Capacity.

SV **:** Sedimentation value.

(V/V)% **:** Percent of volume (of solute) per volume (of solvent).

(W/V)% **:** Percent of weight (of solute) per volume (of solvent).

(W/W)% **:** Percent of weight (of solute) per weight (of solvent).

WEAX **:** Water extractable arabinoxylans.

WUAX **:** Water unextractable arabinoxylans.

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Grand résumé des travaux en Français

Introduction générale

Le blé (Triticum aestivum L.) est à travers l'histoire l'une de nos cultures vivrières les plus importantes et a fourni environ 29,15% des 2600 millions de tonnes de céréales produites dans le monde en 2016 (FAO 2017). Cette importance du blé est due à plusieurs raisons : il est cultivé dans presque toutes les régions du monde. Ainsi, il représente la principale source de revenus pour des millions de petits agriculteurs vivant dans les pays en développement du monde entier. De plus, le blé peut être stocké pendant de longues périodes afin de produire différents types d'aliments à base de blé, fournissant ainsi une source de nutrition abordable à une grande partie de la population mondiale. Ces qualités décrites ont fait du blé une culture vivrière importante pour la sécurité alimentaire, et la céréale la plus commercialisée sur le marché international des denrées alimentaires agricoles, affectant l'économie des agriculteurs et d'autres secteurs de la chaîne alimentaire dans le monde entier.

En outre, à cause de la présence des protéines de gluten dans la farine de blé, principale source des propriétés viscoélastiques primordiale pour la panification, la plus grande partie de la farine produite est utilisée pour la fabrication du pain. En conséquence, l'évaluation et la prédiction de la qualité boulangère du blé reste un grand défi que ça soit pour les sélectionneurs ou bien pour les minotiers et les boulangers, qui sont toujours confrontés au défi de choisir la bonne qualité de blé nécessaire à leur intérêt spécifique et afin de remplir les attributs requis par les consommateurs (Lindhauer, 2014). En effet depuis de nombreuses décennies, un système de méthodes appropriées a été établi pour évaluer les performances du blé au cours de la panification. Ces méthodes sont classifiées en tant que méthodes directes et méthodes indirectes. Les méthodes directes représentent les tests de panification, qui sont toujours considérés comme le moyen le plus précis pour évaluer la qualité boulangère du blé, à travers la détermination de diverses propriétés, entre autres le volume du pain qui est généralement considéré comme le facteur unique sur lequel repose l'évaluation. Cependant, les tests de panification doivent être effectués par un personnel expérimenté et requièrent des équipements spécifiques de boulangerie, du temps et une quantité considérable de blé, ce qui est une condition inappropriée aux premiers stades de la sélection.

Par conséquent, un large éventail de méthodes indirectes a été établi pour caractériser les propriétés des variétés de blé, et par la suite évaluer leur qualité boulangère. Parmi ses méthodes, la teneur en protéines du blé, reste globalement le principal critère sur lequel cette évaluation est fondée, à cause de la relation linéaire qui lie cette teneur avec le volume du

pain (Wrigley et Bietz, 1988). Néanmoins, au cours des dernières années, certaines variétés de blé approuvées en Allemagne ne présentent pas une dépendance linéaire entre la teneur en protéines et le volume du pain. Ces variétés ont des teneurs en protéines de grains relativement faibles ; cependant, en raison de la haute fonctionnalité des protéines du gluten, la qualité boulangère demeure bien mieux que prévu (Lindhauer, 2014). Ces observations mettent en évidence l'impact fondamental de la qualité des protéines du gluten sur la qualité boulangère et les propriétés de la pâte. De ce fait, les paramètres classiques d'évaluation ne semblent plus appropriés pour évaluer la qualité boulangère des variétés allemandes modernes de blé (Seling, 2010, Kazman 2010).

Par conséquent, les études actuelles se concentrent sur l'étude de nouvelles méthodes pour évaluer le volume du pain et la qualité boulangère, sur la base des performances du gluten. Parmi ces méthodes, GlutoPeak®-Test (GPT) a été proposée pour évaluer rapidement la qualité boulangère du blé en mesurant les propriétés d'agrégation du gluten (Kaur et Seetharaman, 2011), tandis que d'autres études portent sur la teneur en macro-polymères du gluten (GMP) (Don et al., 2003a, Graveland et al., 1982, Weegels et al., 1996). Ces deux méthodes nécessitent une très petite quantité de matériel de blé, ce qui serait un intérêt majeur pour les sélectionneurs. Toutefois, seulement quelques études disponibles ont étudié la relation entre le volume du pain et les paramètres mesurés par le GPT, ou bien la teneur en GMP (Thanhaeuser et al., 2014 ; Weegels et al., 1996). En outre, ces études ont été effectuées avec un nombre restreint d'échantillons, ce qui n'a pas pu mettre en évidence l'impact du génotype, de l'environnement et de leur interaction sur l'applicabilité et la capacité des méthodes testées à évaluer la qualité boulangère du blé.

De plus, pour les mélanges de farine de blé, il n'existe pas encore de connaissances scientifiques sur la prédiction de la valeur de l'effet de mélange, qui est un effet synergique notable des mélanges de farine. Cet effet de mélange ne peut être clarifié sans prendre en considération l'influence du génotype, de l'environnement et du niveau de fertilisation.

Ainsi, le but de cette étude est l'évaluation et la prédiction de la qualité boulangère des variétés de blé tendre par le biais de nouveaux paramètres basés sur les propriétés d'agrégation du gluten mesurées par le GPT et sur la concentration en GMP. Cette investigation est réalisée sur la base d'une analyse multi-génotypique et multi-environnementale, afin de mettre en évidence l'impact du génotype et du lieu de croissance sur la capacité de ces méthodes à prédire les performances en termes de panification des variétés de farine de blé tendre.

En plus de ces deux méthodes (GPT et GMP) préalablement mentionnées, les propriétés rhéologiques de la pâte des échantillons de farine de blé ont été aussi déterminées par l'Extensographe. Ces tests ont visé l'évaluation de l'effet de mélange des mélanges de farines de blé tendre et ensuite la prédiction du volume de pain. Cet effect de mélange pourra étre d'un avantage économique pour les agriculteurs et aidera à réduire les effets environnementaux associés à la fertilisation azotée.

Pour atteindre ces objectifs, la méthode GPT a été optimisée en faisant varier le rapport solvant / farine et en utilisant différents solvants comme alternative à l'eau pure afin de tester une série de variétés de blé d'hiver récoltées à 8 sites différents en Allemagne et issue de deux années de récolte. Par la suite, la fraction de GMP de deux séries de variétés de blé d'hiver récoltées à 8 endroits différents en Allemagne à partir des années de récolte 2013 et 2014 a été extraite sous des conditions réductrices et quantifiés en utilisant la méthode HPLC. Enfin, une série de six variétés de blé tendre, récoltées à trois endroits en Allemagne et avec trois niveaux de fertilisation azotée, ont été mélangées avec rapport de 50% / 50%, et testés par le moyen de GPT, GMP et Extensographe.

En conséquence, la thèse est organisée en trois parties principales :

1. Prévision de la qualité boulangère du blé tendre à l'aide d'une méthode optimisée de GlutoPeak®.

2. Relation entre le macro-polymère de la gluténine et la qualité boulangère du blé tendre sur la base d'une analyse multi-environnementale et multi-génotypique.

3. Prédiction de l'effet de mélange et de la qualité boulangère des mélanges de blé tendre au moyen d'Extensographe, de GlutoPeak®-Test et du macro-polymère de gluten dans des conditions de fertilisation et d'environnement variables.

Chapitre 1 : Revue bibliographique

I. Généralités sur le blé

Divers travaux ont été effectués pour discerner les origines du blé, et l'hypothèse la plus plausible postule que l'Homme a commencé à utiliser le blé pour la nourriture à l'époque préhistorique, au moins 15 000 ans avant notre ère. Pourtant le blé que nous consommons aujourd'hui diffère du blé consommé dans les périodes précédents.

Le blé de nos jours est le résultat d'une série de croisements naturels, de mutations et de sélection naturelle, et sa génétique approuve que son développement soit très complexe (Symko, 1999). Le blé est une espèce polyploïde et appartient au genre Triticum. Son évolution est passée par les différentes formes de blé cultivé : un diploïde ; Triticum monococcum (engrain), tétraploïdes ; Tritium turgidum, Triticum dicoccum (amidonnier) et Triticum durum (blé dur) et les espèces hexaploïdes ; Triticum spelta (épeautre) et Triticum aestivum (blé tendre), qui détient trois paires de génomes de chacun des sept chromosomes de base (AA, BB et DD, 2n = 42) (Gustafson et al., 2009 ; McIntosh, 2004). De nos jours, l'engrain et l'amidonnier font parties des grains anciens et sont encore cultivés et utilisés pour l'alimentation du bétail ou bien pour la fabrication de quelques types de bière. Le blé dur est principalement utilisé pour la fabrication des pâtes alimentaires, tandis que le blé tendre, qui est le plus largement cultivé aujourd'hui, est utilisé pour fabriquer un grand éventail de produits à base de pates.

Depuis l'aube de l'agriculture, le blé est à la base de l'alimentation de l'Homme grâce à son adaptabilité à une large gamme de latitudes, sa facilité de conservation, sa qualité nutritionnelle et sa capacité à produire une grande gamme d'aliments appétissants et satisfaisants. Le blé a également gagné de l'importance à cause de son caractère de battage libre qui rendait le grain facile à récolter.

Tout au long de l'histoire, le blé a été une composante importante du régime alimentaire de l'Homme en raison de son adaptabilité agronomique, sa facilité de conservation, sa qualité nutritionnelle et sa capacité à produire une variété d'aliments appétissants, intéressants et satisfaisants. Le blé a également gagné en importance en raison de son caractère grain nu qui rendait le grain facile à récolter.

II. Classification et anatomie du blé

Les différents types de blés sont classifiés selon trois traits principaux : la dureté du grain, la couleur du grain et la saison de croissance.

Selon la dureté du grain, on distingue deux types de blé : le blé tendre et le blé dur. Cette dureté est liée à l'énergie nécessaire pour broyer les grains de blé et les transformer en farine. La mouture du blé dur nécessite plus de force et une énergie plus élevée, par rapport au blé tendre (Atwell, 2001, Feiz et al., 2008). Conformément à la couleur de la graine, le blé est classé comme blé rouge et blanc. Cette couleur dépend de la présence ou de l'absence d'un pigment rougeâtre dans les couches externes du grain de blé, et qui peut être révélé par un

examen visuel (Atwell, 2001, Bechtel et al., 2009). Et enfin sur la base de la saison de croissance, on distingue deux types de blé : le blé d'hiver et le blé de printemps. Le blé d'hiver est semé en automne, subi une période de froid connue sous le nom de vernalisation pour passer du stade végétatif au stade reproductif croitre au printemps. Ce processus de vernalisation se produit généralement à des températures < 8 ° C, et est exigé pour le blé d'hiver afin d'assurer une bonne floraison et une bonne reproduction des graines. Le blé de printemps est planté au printemps et n'a pas besoin de vernalisation pour la floraison. Les deux types de blé sont récoltés en été, tandis que le blé de printemps peut également être récolté en automne.

Le grain de blé est une structure complexe, à maturité formée de trois sections anatomique principales : le son, l'albumen et l'embryon (Atwell, 2001).

Le son est la couche extérieure dure qui protège le grain de blé, et constitue environ 14% du poids total du grain. Le son est composé de deux éléments principaux : le péricarpe et le tégument (Atwell, 2001, Bechtel et al., 2009, Delcour et Hoseney, 2010). Le péricarpe est la couche externe du grain, composé d'épiderme, de couche sous-épidermique, de parenchyme, de cellules transversales et de cellules tubulaires. Ce péricarpe constitue la paroi de l'ovaire mûri, qui adhère au tégument à maturité. Le tégument a également un impact crucial sur le grain de blé, de sorte qu'il contrôle le transfert d'eau entre la graine et son environnement extérieur (Evers et Bechtel, 1988).

L'albumen représente 80 à 85% du poids total du grain, et est le principal constituant de la farine. Ce composant est situé au centre du gain et formé par des cellules qui enferment les particules d'amidon enchâssés dans une matrice protéique (Delcour et Hoseney 2010, Evers et Millar, 2002). L'albumen est la source d'énergie et de protéines nécessaires pour le développement des plants de blé en phase de germination (Atwell, 2001, Bechtel et al., 2009, Delcour et Hoseney, 2010). Entre le son et l'albumen, se situe une couche formée par les cellules de l'albumen, appelée couche a aleurone. Cette couche est principalement éliminée lors de la mouture en raison de sa localisation dans le grain de blé (Atwell, 2001, Bechtel et al., 2009, Delcour et Hoseney, 2010).

Enfin, l'embryon représente environ 3% du poids du grain et contient la plupart des nutriments essentiels pour le grain (Atwell, 2001). Le germe est formé de deux composants principaux : l'axe embryonnaire et le scutellum. L'axe embryonnaire représente la racine et la tige rudimentaires, tandis que le scutellum est un organe de stockage qui transporte les

nutriments de l'endosperme à l'embryon pendant la germination (Bechtel et al., 2009, Evers et Bechtel, 1988, Posner et Hibbs, 1997).

III. Composition du grain de blé

Le blé est constitué essentiellement de glucides (amidon et polysaccharides non amylacés), protéines, lipides et d'autres constituants mineurs ; vitamines et minéraux (Koehler et Wieser, 2013, Shewry et al, 2010).

1. Glucides

L'amidon représente 60% à 70% de la matière sèche totale (MS) du grain du blé, constituant la réserve alimentaire fondamentale du grain. L'amidon se trouve dans l'albumen sous la forme de granules stockés dans les amyloplastes. Ces granules d'amidon sont constitués de deux types de polymère de glucose qui sont l'amylose (environ 20-30%) et l'amylopectine (environ 70-80%) (Konik-Rose et al., 2007). L'amylose est formé de longues chaînes linéaires de monomères de D-glucopyranose reliées par des liaisons glycosidiques en α-(1-4) et son degré de polymérisation est compris entre 500 et 6 000 résidus de glucose (Hizukuri et al., 1981 ; Shibanuma et al., 1994). Cependant, l'amylopectine est une molécule ramifiée de degré de polymérisation plus élevé (3×10^5 a 3×10^6), constituée de chaînes principales de monomères de D-glucopyranose reliées par des liaisons glycosidiques en α-(1-4), sur lesquelles des chaînes latérales identiques viennent se brancher par des liaisons α-(1-6) (Lineback and Rasper 1988).

D'autres types de glucides sont présents dans le grain de blé et sont principalement des constituants des parois cellulaires de l'albumen, connus sous le nom de polysaccharides non amylacés, et de fibres alimentaires du point de vue nutritionnel (≈ 10% MS du grain). Les principaux types de polysaccharides non amylacés sont les arabinoxylanes, la cellulose, les β-glucanes et les arabinogalactanes (Delcour et Hoseney, 2010).

Les arabinoxylanes (AX) sont les composés majoritaires des pentosanes (85 à 90%) (Delcour et Hoseney, 2010 ; Koehler et Wieser, 2013). Ce sont des hétéropolymères constitués d'une chaîne linéaire de xylose associés par des liaisons β-(1-4), sur lesquels sont greffées des substitutions d'arabinose distrubuées irrégulièrement et auxquelles l'acide férulique peut être lié de manière covalente par des liaisons ester (Meuser et Suckow, 1986). En fonction de leur solubilité dans l'eau, les AX peuvent être subdivisés en deux catégories : les AX solubles et AX insolubles (Delcour et Hoseney, 2010, Saulnier et al., 2007). L'insolubilité des AX

s'expliquerait par un poids moléculaire des AX insolubles (100-530 kDa) plus élevé que celui des AX solubles (20-120 kDa) et par un enchevêtrement des molécules plus important (Delcour et Hoseney, 2010, Stone et Morell, 2009). Outre les arabinoxylanes, le pentosane contient également une autre fraction soluble dans l'eau, qui est l'arabinogalactane. Cette fraction est hautement ramifiée, constituée de motifs de galactopyranose liés en β-(1-3) et β-(1-6) avec des résidus d'arabinofuranose liés aux glycosides (Kohler et Wieser, 2013).
La cellulose est également un polymère linéaire constitué de plusieurs unités de glucopyranose liées par des liaisons glycosidiques β- (1-4), formant des chaînes polysaccharidiques. Les chaînes sont réunies dans une structure tridimensionnelle et stabilisées par des liaisons hydrogène intermoléculaires et des interactions de van der Waals (Delcour et Hoseney, 2010, Stone et Morell, 2009).
Enfin, les (1-3) - (1-4) -β-glucanes appelés « béta-glucanes » sont constitués d'une chaîne linéaire de D-glucose liée par des liaisons glycosidiques β- (1-4) et β- (1-3) (Woodward et al., 1983, Li et al., 2006).

2. Lipides et constituants mineurs

Les lipides du blé représentent environ 3% du poids total des grains et se retrouve principalement dans le germe, et dans une moindre mesure dans l'albumen et la couche à aleurone (Koehler et Wieser, 2013). Les lipides non liés à l'amidon constituent environ 75% des lipides du blé et sont classés comme étant libres, solubles dans le pétrole ou l'éther diéthylique, ou liés, solubles uniquement dans des mélanges de solvants polaires froids (MacMurray et Morrison, 1970). Les lipides libres peuvent en outre être classés en une fraction lipidique polaire (glycolipides et phospholipides) et non polaire (principalement triglycérides). Les lipides liés sont constitués d'une partie des phospholipides et des glycolipides qui peuvent être associés, par exemple avec des protéines (Delcour et Hoseney, 2010). Les lipides liés à l'amidon sont également classés comme non polaires et polaires (Delcour et Hoseney, 2010 ; Wrigley et al., 2009). Les triglycérides sont la classe dominante des lipides dans le germe et dans la couche à aleurone, tandis que les phospho- et glycolipides sont présents dans l'albumen (Hoseney, 1994).

La teneur en minéraux du grain de blé varie entre environ 1,0 et 2,5%, et est fortement liée au génotype, à la composition du sol, aux conditions climatiques et aux apports externes (fertilisation) (Koehler et Wieser, 2013 ; Bock, 2000). La plus grande partie des minéraux (> 90%) se trouve dans le son, la couche à aleurone et le germe, par conséquent, le processus de

mouture peut entraîner des pertes importantes de minéraux (Koehler et Wieser, 2013). Le magnésium, le phosphore et le potassium sont présents dans le blé avec des quantités significatives, par rapport aux autres minéraux ; le magnésium, le phosphore et le potassium (Davis et al., 1984).

Le grain de blé contient aussi des vitamines dune concentration allant de moins de 1 à environ 50 mg / kg (Koehler et Wieser, 2013) et demeure une bonne source de vitamines B (thiamine, riboflavine, niacine et pyridoxine), ainsi que des vitamines liposolubles (tocophérols). A l'instar des minéraux, ces vitamines sont concentrées notamment dans la couche à aleurone et dans le germe, ce qui rend leur élimination éventuelle lors de la mouture.

3. Protéines

Les protéines forment de 9 à plus de 15 % de la matière sèche du blé et sont principalement concentrées dans l'albumen, qui est considéré comme principale source de farine blanche obtenue après mouture des gains et tamisage du blé (Belitz et al., 2009).
Elles ont été classées par Osborne (1907) en quatre groupes en fonction de leur solubilité dans différents solvants : Le groupe des albumines, composé des protéines solubles dans l'eau; le groupe des globulines, composé des protéines solubles dans les solutions salines diluées ; le groupe des gliadines, composé de protéines solubles dans les alcools dilués de type éthanol 60 % - 70 %; et enfin le groupe des gluténines, composé de protéines insolubles dans les solvants précédents et partiellement solubles dans les solutions acides et basiques diluées. Cependant, la classification d'Osborne exclue une fraction notable de gluténines, qui est insoluble dans les acides dilués et son extraction au moyen de solutions basiques fortes détruit sa structure primaire (Koehler et Wieser, 2013). En outre, la classification d'Osborne ne fournie pas une séparation nette des protéines de blé sur la base des caractéristiques biochimiques et fonctionnelles, et demeure est définie fortement influencée par les conditions expérimentales. Pourtant, cette méthode reste souvent très utile en tant qu'étape de séparation initiale pour obtenir des fractions protéiques semi-pures (Goesaert et al., 2005).
Par conséquent, les protéines de blé sont également classées en protéines fonctionnelles (non-gluten) et protéines du gluten (Shewry, 2003). Les protéines fonctionnelles sont principalement les albumines et globulines, et couvrent entre 15 et 20% des protéines d'un grain (Goesaert et al., 2005, Delcour et al., 2012). Les protéines du gluten (entre 80 et 85% des protéine de blé) sont constituées de gliadines et de gluténines et sont connues sous le nom

de protéines de réserve parce qu'elles fournissent de l'azote et des acides aminés aux plants de blé au cours de la germination (Shewry et al., 1995 ; Shewry et Halford, 2002).

Les albumines et les globulines ont une masse moléculaire inférieure à 25 000 Da (Veraverbeke et Delcour, 2002). L'alpha-amylase / trypsine, les serpines et les purothionines sont les albumines et les globulines prédominantes dans le grain de blé (Østergaard et al., 2000, Garcia-Olmedo et al., 2002). En général, ce sont des protéines monomériques ayant généralement un rôle physiologique et structural (Goesaert et al., 2005), pourtant leur rôle en panification reste non clair (Veraverbeke et Delcour, 2002).

Les gliadines sont des protéines monomèriques constituées de polypeptides d'une masse moléculaire comprise entre 30 000 et 75 000 Da (D'Ovidio et Masci, 2004, Goesaert et al., 2005, Delcour et al., 2012). Ils représentent environ 40 à 50% de la teneur totale en protéines du blé et sont solubles dans l'éthanol à 70% (v / v) (Qi et al., 2006 ; Thewissen et al., 2011). Biochimiquement, les gliadines sont classées en quatre sous-types : alpha- (α-), bêta- (β-), gamma- (γ-), et les omega- (ω-) gliadines (Shewry et al., 1986). Étant donné que les masses moléculaires des α- et β-gliadines sont similaires, ils sont souvent appelés gliadines de type α et, par conséquent, de nombreux auteurs ont classé les gliadines en trois groupes seulement : les types α, γ et ω (Kuktaité, 2004). Les gliadines sont riches en proline et en glutamine et contiennent un nombre élevé de résidus de cystéine (Shewry, 2003), par conséquent ils ont été classés en tant que ; protéines riches en soufre (S). Les gliadines de type α possèdent six résidus alors que les γ gliadines contiennent huit résidus de cystéine engagés dans des ponts disulfures. Cependant, les ω-gliadines sont sont caractérisés par l'absence totale des cystéines (protéines pauvres en S). De ce fait, les ω gliadines sont incapables de participer à l'établissement du réseau protéique par la formation des liaisons covalentes de types disulfures (S-S) (Delcour et al., 2012, Lafiandra et MacRitchie, 1997, Wieser, 2007). Durant la formation de la pâte, les gliadines hydratés ne peuvent pas être liées par covalence et former de grands réseaux élastiques comme les gluténines, mais elles agissent comme un plastifiant et contribuent principalement à la viscosité et l'extensibilité de la pâte, qui sont des caractéristiques rhéologiques importantes de la pâte. Les gliadines peuvent interagir par des interactions hydrophobes et des liaisons hydrogène et s'associer avec d'autres gliadines ou gluténines (Veraverbeke et Delcour, 2002).

Les gluténines sont constituées de deux types de sous-unités : sous-unités de gluténine à masse moléculaire élevée (HMW-GS), représentant environ 10% de la teneur totale du gluten et les sous-unités de gluténine de faible masse moléculaire (LMW-GS) avec environ 20% de la teneur totale du gluten. Les HMW et les LMV ont une nasse moléculaire qui se situe entre

80 000 et 120 000 et entre 30 000 et 55 000, respectivement (Lafiandra et MacRitchie, 1997 ; Southan et MacRitchie, 1999 ; Delcour et al., 2012). Ces deux fractions sont obtenues après réduction des gluténines au moyen d'un réducteur disulfure tel que le dithiothréitol (DTT) (Delcour et al., 2012, Lafiandra et MacRitchie, 1997). Ensuite, selon leur profil de mobilité électrophorétique, les HMW-GS et LMW-GS sont classés en quatre groupes (régions A-B-C-D). La région A correspond à HMW-GS, tandis que les régions B, C et D se rapportent aux gluténines LMW (Delcour et al., 2012, Wrigley, 1996, Gianibelli et al., 2001). Les séquences d'acides aminés de LMW-GS dans les régions C et D sont similaires à la séquence d'acides aminés de (α ; γ) et ω gliadines, respectivement (Gianibelli et al., 2001). Selon Delcour et al, 2012, la principale différence entre les régions B, C et D est le premier résidu d'acide aminé qui forme leur structure, qui peut être la sérine, la méthionine ou l'isoleucine. Deux types de HMW-GS sont à distinguer : le type x-HMW-GS et le type y-HMW-GS (Delcour et al., 2012). Le type x-HMW-GS possède trois résidus de cystéine, alors que le type y-HMW-GS en possède cinq. Ces propriétés permettent la formation des liaisons disulfures intra- et intermoléculaires, conférant à HMW-G son caractère polymérique, et permettant de relier HMW-GS et LMW-GS via formant des réseaux polymériques avec des masses moléculaires de plusieurs millions (Lafiandra et MacRitchie, 1997). En raison de cette capacité de polymérisation, le poids moléculaire de la gluténine peut varier de 100 000 Da jusqu'à des millions de Da (Schofield et al 1983).

Le plus grand de ces polymères de gluténines est appelé gluténine macropolymère (GMP). GMP est constitué de très grandes structures de HMW et LMW, liées par des liaisons disulfures intra- et intermoléculaires (Don et al., 2003a), avec un poids moléculaire estimé de plusieurs millions de Da ($> 5 \times 10^6$) (Schofield et coll., 1983, Weegels et coll., 1996, Koehler, 2010). Le GMP est insoluble dans la solution de dodécylsulfate de sodium (SDS) et forme une couche de gel sur le culot d'amidon résultant après centrifugation de la farine de blé tamponnée dans une solution de SDS (Graveland et al., 1979). Des études précédentes ont rapporté le rôle significatif du GMP et de ses sous-unités HMW et LMW ainsi que le rapport LMW / HMW dans l'évaluation de la qualité de la farine de blé, de la qualité boulangère et des propriétés de la pâte (Sapirstein et Suchy, 1999 ; Thanhaueser et al. 2014).

IV. Effet de la génétique et de l'environnement sur les protéines du blé

De nombreuses études ont signalé l'impact du contexte génétique, des conditions environnementales et leurs interactions sur les protéines de blé. Les conditions environnementales sont principalement la température, la disponibilité en eau au lieu de

croissance du blé ainsi que la fertilisation azotée. D'après Weegels et al., 1996 ; Johansson et Svensson, 1998, le contexte génétique influence fortement la composition et la teneur en protéines du blé, ce qui affecte aussi les caractéristiques de la pâte et la qualité boulangère. Cet effet a été expliqué par le nombre des résidus de cystéine qui diffère entre les différentes variétés de blé (Lindsay et Skerritt, 1998). Ce résultat a été confirmée par les études de Shewry et al., 1992 ; Johansson et Svensson, 1999, qui soulignent que la présence de l'allèle 1Dx5 de HMW-GS conduit à une meilleure qualité boulangère, grâce à sa composition riche en résidus de cystéine par rapport à l'allèle 1Dx2.

Les conditions environnementales caractérisant le lieu de croissance ont également une influence significative sur la teneur en protéines et en sous-unités protéiques ainsi que la taille des protéines polymériques (Johansson et al., 2001, 2002, Dupont et Altenbach, 2003). Selon (Hall, 2001) et (Jaleel et al., 2009), le stress thermique peut causer des dommages irréversibles aux au développement de la plante, alors que le stress hydrique affecte principalement la quantité et la qualité des protéines par le raccourcissement de la phase de maturation des grains. Blumenthal et al. (1995) rapporte que le stress thermique (> 35 ° C) induit une augmentation du rapport gliadines / gluténines et une diminution de la proportion en gros polymères dans la farine de blé. Cependant cet effet reste dépendant du contexte génétique, tel que les variétés ayant les allèles 1Dx5 et 1Dy10 pour HMW-GS ont généralement montré plus de stabilité en réponse à une température élevée en comparaison avec les variétés possédant les allèles 1Dx2, 1Dy12 (Blumenthal, 1995 ; Panozzo et Eagles, 2000). Irmak et al., 2008).

D’autre part la fertilisation azotée joue un rôle crucial que ce soit pour le rendement ou bien pour la synthèse des protéines. Normalement, la fertilisation azotée du blé est divisée en trois applications au début des stades de croissance du blé végétal pour assurer un rendement grainier adéquat, suivi généralement d'une fertilisation azotée tardive afin d'augmenter la teneur en protéines du grain (Olson et Kurtz, 1982). Comme démontré précédemment, la fertilisation azotée n'influence pas seulement la quantité de protéines (Fuertes-Mendizabal et al., 2010 ; Garrido-Lestache et al., 2005 ; Wieser et Seilmeier, 1998), mais aussi leur qualité (Johansson et al., 2001). Avec l'augmentation du niveau de fertilisation, la teneur en protéines du gluten augmente, cependant les effets sur les gliadines sont plus prononcés que sur les gluténines, ce qui entraîne un rapport élevé gliadine / gluténine (Martre et al., 2006). Particulièrement selon les études de Wieser et Seilmeier, 1998, une forte fertilisation azotée induit l’augmentation des proportions de ω-gliadines et de HMW-GS, et en revanche une diminution des proportions de LMW-GS et de γ-gliadines, alors que les quantités d'albumine

et de globuline restent constants. Cependant, l'effet de la fertilisation azotée tardive varie selon les conditions de croissance, l'année de récolte, le moment de la fertilisation et le type d'engrais azoté utilisés (Fuertes-Mendizabal et al., 2010, 2013 ; Garrido-Lestache et al., 2004).

V. La fonctionnalité du gluten dans le processus de panification

Apres mouture des grains de blé, la farine résultante est mixée avec d'autres ingrédients nécessaires à la panification, et soumise à une action mécanique. En présence d'hydratation et de cette énergie mécanique, les gliadines et les gluténines forment un réseau tridimensionnel viscoélastique appelé gluten, qui représente la masse caoutchouteuse restante lorsque la farine de blé est lavée à l'eau pour éliminer l'amidon, les polysaccharides non amylacés et les constituants solubles dans l'eau. Les gliadines et les gluténines confère au gluten ses propriétés d'élasticité et d'extensibilité qui sont essentielles pour la qualité des farines de blé (Shewry et al., 1995). Grâce à ces propriétés, la farine de blé peut être transformée en une grande gamme de produits alimentaires : pains, pâtes alimentaires, pâtisseries et de nombreux autres aliments (Day et al., 2006). Par conséquent, les protéines du gluten subissent plusieurs changements au cours des différentes étapes du processus de fabrication du pain.

L'apport d'eau et d'énergie mécanique au cours du mélange des ingrédients nécessaires à la panification au cours du pétrissage modifient la conformation initiale des chaînes de gliadine et de gluténine (Wieser, 2007 ; Schiedt et al., 2013). Lorsque l'eau est ajoutée à la farine de blé, les protéines du gluten se dénaturent, favorisant la distribution hétérogène de l'eau autour des longues chaînes protéiques, notamment autour des acides aminés polaires et des groupes ionisables (Schiedt et al., 2013). Alors que les forces de cisaillement et de traction subies au cours du pétrissage permettent aux protéines de gluten de se rejoindre et former un réseau complexe, appelé gluten (Singh et MacRitchie, 2001). La transformation des protéines du gluten (gliadines et gluténines) en un réseau de gluten viscoélastique cohésif continue se produit lorsque les liens natifs de ces protéines sont brisés et de nouvelles liaisons se forment, donnant une matrice protéique beaucoup plus forte que les agrégats de protéines préalablement présents dans la farine blé avant hydratation (Schiedt et al., 2013). Selon Shewry et al. (2002), Delcour et al. (2012), et Belton (1999), le réseau du gluten est stabilisé par des interactions intra- et intermoléculaires, qui consistent principalement en liaisons disulfure, ainsi que des interactions non covalentes, en particulier les liaisons hydrogène, les interactions hydrophobes et l'interaction électrostatique. Ces liaisons formées fournissent des caractéristiques d'extensibilité et d'élasticité. Tel que rapporté par Shewry et al., (2002) ; Don

et al., (2003a, b) ; Wieser (2007); Wang et al., (2015), l'élasticité du réseau de gluten est principalement attribuée aux liaisons disulfure entre les chaînes individuelles de gluténines. Ainsi, les polymères de gluténine sont connus par leur aptitude à générer un réseau continu qui fournit de la résistance (résistance à la déformation) et de l'élasticité à la pâte (Belton, 1999), tandis que les gliadines monomères agissent comme plastifiants du système polymérique (Cornec et al., 1994 ; Khatkar et al., 1995).

Pendant la fermentation, le réseau du gluten formé joue un rôle majeur dans la rétention du dioxyde de carbone occlus dans les bulles de gaz empêchant leur coalescence. La capacité de rétention du gaz détermine le volume et la structure de la mie du pain obtenu (Cauvain 2015, Hamer et al., 2009).

Au cours de la cuisson du pain, d'autres changements notables se produisent au niveau du réseau du gluten et qui sont dues à l'apport d'énergie thermique. La coagulation ou bien le repliement des protéines formant le réseau du gluten reste la transformation primordiale engendrée pendant la cuisson. A ce stade, la solubilité du gluten diminue, indiquant une polymérisation et une réticulation accrues du polymère de gluten, qui est attribuée à des réactions d'échanges de groupes disulfures (Schofield et al, 1983). Cette réticulation du gluten est aussi indiquée par la diminution de l'hydrophobicité de la surface du gluten qui subit également des changements, tout en entrainant une exposition des groupes hydrophobes et une diminution de la solubilité (Guerrieri et al., 1996). Ces réactions déterminent la structure finale du gluten (perte d'élasticité), du volume du pain et de ces caractéristiques.

VI. La qualité boulangère du pain

Tel que rapporté par Finny et al. (1948), la qualité boulangère du blé et la teneur en protéines et en gluten sont liés de façon linéaire. Cependant, cette relation dépend de chaque variété de blé, ce qui suggère que la qualité des protéines est également un critère majeur qu'il faut prendre en considération. Ainsi, la quantité et la qualité des protéines de gluten sont des caractéristiques importantes pour la fabrication du pain.

La qualité du gluten est supposée être déterminée par le rapport gliadines / gluténines et la qualité de la fraction gluténines (Goesaert et al., 2006). Les propriétés des gluténines (composition, structure et distribution de la taille moléculaire) s'avèrent avoir un impact crucial sur les différentes qualités du gluten des différentes variétés de blé (Goesaert et al., 2006). L'existence de plus de 20 différents sous unités de HMW-GS et de plus de 40 de

LMW-GS engendre différentes compositions et structures des gluténines. Ceci induit des différences au niveau des interactions non covalentes qui déterminent l'élasticité de réseau du gluten, ce qui affecte largement les fonctionnalités de ces protéines au cours de la panification (Gupta et Shepherd, 1990, Goesaert et al., 2006). Cette propriété d'élasticité apparait aussi être conditionnée par la présence de spécifiques polymères de gluténines possédant une certaine distribution de masse moléculaire (MacRitchie, 1992 ; Singh et MacRitchie, 2001). Ce résultat a été confirmé par d'autres études (Singh et MacRitchie, 2001 ; Veraverbeke et Delcour, 2002) mettant en relief des corrélations positives entre la résistance de la pâte et les teneurs en macro- polymères de gluten, connu comme étant le plus grand polymère de la fraction gluténique insoluble au SDS.

Chapitre 2 : Matériel et méthodes

1. Matériel végétal

Tous les échantillons étudiés dans la présente thèse sont des variétés de blé tendre d'hiver (Triticum aestivum L.) et sont toutes des variétés commerciales qui sont actuellement inscrites sur la liste descriptive de variétés allemandes (Beschreibende Sortenliste (BSL) vom Bundessortenamt).

Dans le premier lot de travail de cette thèse, le matériel utilisé est composé de :

- 15 différentes variétés de blé tendre de l'année de récolte 2013.
- 11 différentes variétés de blé tendre de l'année de récolte 2014.
- 21 différentes variétés de blé tendre de l'année de récolte 2015.

Chaque variété est cultivée dans huit endroits différents en Allemagne, qui représentent les principaux lieux de croissance du blé allemand, caractérisés par des conditions pédologiques et climatiques différentes. Le matériel végétal étudié dans cette première partie était donc constitué de 120, 88 et 208 différents échantillons de farine de blé tendre provenant, respectivement, des années de récolte 2013, 2014 et 2015.

Pour le deuxième lot de travail, les mêmes variétés utilisées dans le premier lot de travail ont été considérées, à l'exception des variétés de blé tendre provenant de l'année de récolte 2015.

Enfin, pour le troisième lot de travail, six variétés différentes de blé tendre d'hiver de l'année de récolte 2015, cultivées à trois endroits différents en Allemagne (Basse-Saxe et Saxe-Anhalt) et avec trois niveaux différents de fertilisation azotée (faible, moyen et haut niveau de

fertilisation) ont été étudiés. Ces variétés ont reçu la même quantité d'engrais azoté au début de la végétation (50 kg N / ha), mais différents niveaux de fertilisation azotée tardive, niveau faible (160 kg N / ha), niveau moyen (180 kg N / ha) et niveau élevé (200 kg N / ha). Ces variétés pures ont été mélangées les unes aux autres, après broyage en farine selon un rapport de 50 :50, ce qui a résulté en 135 échantillons de mélanges de farine de blé et 54 échantillons purs de farine de blé tendre.

Tous les échantillons utilisés dans la présente étude sont classés selon la liste descriptive des variétés allemandes en trois groupes de qualité : blé élite (classe E), blé de qualité (classe A) et blé panifiable (classe B). Seuls ces trois groupes de qualité ont été inclus dans cette étude, tandis que la classe C, qui n'est pas utilisable pour la panification, n'a pas été impliquée.

2. Méthodes expérimentales

Toutes les variétés de blé ont été transformées en farine à l'aide du moulin de laboratoire Buehler MLU 202. La farine de type 550 a été produite conformément à la norme DIN 10355 avec une teneur en cendres d'environ 0,60%, comme recommandé par Arbeitsgemeinschaft Getreideforschung, (2016).

La teneur en cendres a été déterminée selon la méthode ICC 104/1. Les échantillons sont pesés (5 à 6 g) dans des creusets et placés dans un four à moufle à 900 ° C pendant 2 heures. Le résidu est refroidi dans un dessiccateur puis pesé pour être exprimé en pourcentage du poids initial des échantillons.

La teneur en cendres est une mesure extrêmement importante pour atteindre les niveaux de cendres spécifiques des différents types de farine de blé.

2.1. Détermination de la teneur en eau

La détermination de la teneur en eau (teneur en humidité) est une première étape essentielle dans l'analyse de la qualité de la farine de blé, puisqu’elle est utilisée pour d'autres tests. Une teneur en humidité de 14% est couramment utilisée comme facteur de conversion pour d'autres essais dans lesquels les résultats sont influencés par la teneur en eau. De plus, il représente également un indicateur de la durée de conservation du grain. Du blé ou de la farine à forte teneur en humidité, plus de 14,5% attire la moisissure, les bactéries et les insectes, ce qui cause une détérioration au cours du stockage (Centre, 2004).

Dans la présente étude, la teneur en humidité du grain de blé entier a été déterminée selon la norme DIN EN 15948 : 2012 avec la spectroscopie de transmission proche infrarouge (NIT) au moyen de Infratec ™ 1241 (Foss GMbH, Rellingen, Allemagne).

2.2. Détermination de la teneur en protéines brutes

La teneur en protéines brutes a été déterminée en utilisant NIT au moyen de Infratec ™ 1241 (Foss GMbH, Rellingen, Allemagne), conformément à la norme DIN EN 15948 : 2012. Cette teneur est exprimée en pourcentage, sur une base sèche.

La teneur en protéines brutes en tant que paramètre indirect pour l'évaluation de la qualité boulangère reste globalement le principal critère d'évaluation de la qualité boulangère du blé car les protéines du gluten sont reconnues comme les éléments les plus importants de la qualité panifiable (Wrigley et Bietz, 1988). Cependant, la teneur en protéines ne peut pas être considérée comme un paramètre unique pour caractériser la qualité boulangère de la farine de blé, car elle est largement déterminée par la qualité de ses composants protéiques (Caballero et al., 2007).

2.3. Détermination de la valeur de sédimentation

Les propriétés de gonflement des farines de blé sont testées en déterminant la valeur de sédimentation, qui est une mesure approximative de la qualité boulangère. Puisque les protéines du gluten sont insolubles dans l'eau, le volume de sédiment indique la quantité de gluten et reflète les propriétés de gonflement du gluten. Cela signifie que la valeur de la sédimentation en tant que paramètre indirect pour évaluer la qualité boulangère du blé combine des mesures qualitatives et quantitatives du gluten de blé (Freund et Kim, 2006).

La valeur de Zeleny de sédimentation a été déterminée selon ICC 116/1, après préparation des échantillons de farine selon ICC 118. La valeur de sédimentation variera de 8 ml pour une teneur en protéines très faible (gluten faible), à environ 78 ml pour une teneur en protéines très élevée et un type de gluten fort.

2.4. Test de panification : Rapid Mix Test

Le test de panification utilisé dans la présente étude est le test Rapid Mix (Pelshenke et al., 2007) ; considéré conne une méthode de panification standard pour produire des petits pains afin de mesurer le volume du pain. Le nom de ce test de cuisson est dû à la rapidité qui caractérise cette méthode en termes de mélange et de fabrication de petits pains. De plus, cette

méthode est basée sur l'automatisation de la plupart des activités, afin de limiter l'influence humaine sur les résultats (Freund et Kim, 2006).

Avant d'entamer ce test de panification, il faut déterminer la teneur en humidité de la farine de blé utilisée selon DIN EN 15948 : 2012, l'indice de chute selon Hagberg-Perten DIN EN ISO 3093 : 2009 et enfin l'absorption de l'eau par le moyen du Farinograph et selon ICC 115/1.

2.5. GlutoPeak®-Test

L'évaluation des propriétés d'agrégation du gluten a été effectuée en utilisant le GlutoPeak®-Test (GPT) (Brabender GmbH et Co KG, Duisburg, Allemagne).

Afin d'optimiser la méthode GPT standard et permettre une séparation entre les variétés étudiées, différents solvants ont été utilisés et différentes quantités de farine et de solvant ont été ajoutées dans la coupe à 36 ° C, et la palette de mélange a été réglée à une vitesse de 2750 tr/min.

Les solvants utilisés sont : eau distillée, solution de saccharose à 5,0 %, solution de carbonate de sodium à 5,0 %, solution d'acide lactique à 5,0 % et une solution de chlorure de sodium à 1,0 %.

9 g de farine ont été dispersés dans 9 g de solvant dans le cas d'eau distillée, de solution de saccharose, de solution de carbonate de sodium et d'acide lactique, tandis que 10,2 g de farine ont été ajoutés à 11,2 g de solvant dans le cas de chlorure de sodium.

Toutes les mesures ont été effectuées pendant 300 s avec deux répétitions.

2.6. Extraction et quantification du GMP et de ses sous-unités

GMP des échantillons de farine ont été extraits sans des conditions réductrices et quantifiés par le biais de la méthode HPLC suivant la procédure décrite par Thanhaeuser et al. (2014). La procédure d'extraction est résumée sur la figure 4 en tant que représentation schématique.

100 mg de farine ont été pesés dans un tube Pyrex (capacité : 12 ml), puis extraits deux fois à température ambiante avec le solvant d'extraction A (1% de SDS et 0,05 mol / L de NaH2PO4 (pH 6,9)), afin de séparer la fraction de gluténine extractible au SDS. La valeur du pH du solvant A a été ajustée avec HCl (32%).

Cette première extraction a été initiée par une action de vortex pendant 2 min après addition de 1 ml du solvant A, puis suivie d'une agitation magnétique pendant 30 min à température ambiante. Ensuite, une centrifugation de la suspension à 20 ° C pendant 25 minutes à 4600 tr / min a été effectuée (Heraeus Megafuge 1.0, Thermo Fisher Scientific, Dreieich, Allemagne). Le surnageant contenant la fraction de gluténine extractible au SDS a été retiré au moyen d'une pipette et le précipité a été extrait deux fois pour la seconde fois en utilisant le solvant d'extraction B (1-propanol à 50%, 0,05 mol / L HKNaPO4 (pH 7,5) et 1% de DTT).

1 ml du solvant d'extraction B a été ajouté au précipité, qui a été recouvert avec de l'azote pendant 15 secondes. Ensuite, il a été agité au vortex pendant 2 minutes, puis a été soumis à une agitation pendant 30 minutes à 60 ° C dans un bain-marie. Après refroidissement du tube Pyrex dans de l'eau courante froide, la suspension a été centrifugée à 20 ° C pendant 25 minutes à 4600 tr / min. Le surnageant contenant le GMP a été transféré dans une fiole jaugée (capacité : 2 ml) et dilué à 2 ml avec du solvant B. Les extraits ont été filtrés à travers un filtre à membrane d'acétate de cellulose de 0,45 pm et transférés dans des flacons de HPLC.

Un instrument HPLC Kontron (Kontron Instrument, Roßdorf, Allemagne) équipé d'une colonne Yarra 3u SEC-3000 (Phenomenex, Aschaffenburg, Allemagne) a été utilisé pour quantifier les sous-unités de GMP et GMP dans les conditions suivantes: La température de la colonne était la température ambiante, le volume d'injection était 20 µL, l'éluant était le solvant A à un débit de 0,3 ml / min, et la détection a été effectuée en mesurant l'absorbance UV à 210 nm au moyen d'un détecteur Kontron 332 UV-Vis.

L'intégration des zones obtenues a été réalisée à l'aide du logiciel Chromgate (Knauer, Berlin, Allemagne).

Les extractions et les mesures ont été effectuées pour chaque échantillon de farine en deux répétitions.

2.7. La méthode de l’Extensographe

L'Extensographe est considéré comme un équipement utile pour déterminer la résistance et l'extensibilité d'une pâte en mesurant la force nécessaire pour étirer la pâte avec un crochet jusqu'à ce qu'elle se casse. Ce test est réalisé selon la méthode ICC 144/1.

Les résultats de l'extensographe comprennent la résistance à l'extension (R), l'extensibilité (E), l'énergie (En) et le rapport R/E. Ces paramètres sont mesurés à trois reprises, après 45min, 90min et 135 min de fermentation.

Toutes les mesures d'extensographe étude ont été effectuées pour chaque échantillon en deux répétitions.

3. Les méthodes statistiques

Toutes les analyses statistiques ont été réalisées en utilisant SigmaPlot pour Windows version 11 et 14 (Systat Software Inc., San Jose, USA).

Les méthodes statistiques utilisées dans cette thèse sont :

- La corrélation linéaire de Pearson.
- L'analyse de la variance (ANOVA).
- Le test de Kruskal-Wallis.
- Le test de Dunn.
- Le test de Holm-Sidak.
- L'analyse de régression des meilleurs sous-ensembles.

Chapitre 3 : Résultats et discussion

I. Prévision de la qualité boulangère blé tendre à l'aide d'une méthode optimisée de GlutoPeak®-Test.

GPT a été proposé comme une méthode rapide de mesure des propriétés d'agrégation du gluten dans l'objectif de pouvoir rapidement évaluer la qualité boulangère du blé (Kaur et Seetharaman, 2011). En raison de trois éléments clés qui caractérisent cette méthode : nécessité de seulement une petite quantité d'échantillon (3-10g), manipulation facile, résultats en quelques minutes (1-10min), beaucoup d'attention a été donnée à cet équipement, et sa capacité à évaluer la qualité du gluten de blé. Au cours du test du GPT, l'échantillon de farine est mélangé avec le solvant selon un rapport farine / solvant défini, puis soumis à une action mécanique intense, en raison de la vitesse élevée de la palette de mélange. Ces conditions induisent la formation du réseau de gluten et l'augmentation de la consistance de la pâte jusqu'à atteindre une valeur maximale. Ensuite, la contrainte mécanique provoque la dégradation du réseau de gluten, qui est enregistrée comme une diminution de la consistance (Marti et al., 2015a).

Selon les résultats obtenus des études précédentes, les paramètres mesurés au cours du test du GPT montrent une corrélation significative avec les paramètres mesurant la stabilité, l'extensibilité et la ténacité de la pâte (Marti et al., 2015a) et recommandent le GPT comme outil de sélection valide pour la qualité du blé dur. (Marti et al., 2013, 2014). Cependant, les études disponibles n'ont pas encore étudié la relation entre les paramètres du GPT et le volume du pain issu d'échantillons de farine de blé tendre sur la base d'une analyse multi-génotypique et multi-environnemental (plusieurs lieux de croissance), puisqu'ils ont testé la méthode GPT avec un nombre restreint d'échantillons. Néanmoins, le génotype, le lieu de croissance et leur interaction ont un effet important sur l'évaluation d'une nouvelle méthode, car les différentes composantes des protéines de la farine diffèrent en fonction de l'environnement et du génotype et changeront en fonction du lieu, des conditions culturales et de la saison de croissance. Akcura, 2014).

Par conséquent, le premier objectif de cette étude a été l'optimisation de la méthode standard du GPT, tout en utilisant différents solvants et en faisant varier le rapport solvant / farine, tandis que la température a été portée à 36 ° C, et la palette de mélange a été réglée à une vitesse de 2750 tr / min. Les solvants utilisés sont : eau distillée, solution de saccharose à 5,0 %, solution de carbonate de sodium à 5,0 %, solution d'acide lactique à 5,0 % et une solution de chlorure de sodium à 1,0 %.

Parmi ces solvants utilisés, l'eau distillée et la solution de saccharose ont généré la formation du réseau de gluten qui a été clairement démontrée par les couples obtenus par le GPT. Au contraire la solution de carbonate de sodium n'a pas généré de formation de pic. Cette observation peut être attribuée à l'effet du pH sur les protéines de blé, qui peut induire une modification de la structure des protéines par attraction ou répulsion électrostatique résultant des changements du degré d'ionisation des groupes ionisables des protéines de la farine. En présence de carbonate de sodium, le pH de 11,6 induit de fortes forces de répulsion entre les charges hautement négatives des chaînes protéiques du gluten, qui pourraient empêcher les molécules de ces protéines de s'associer et de former des réseaux. Cependant, les trois solvants préalablement mentionnés n'ont pas permis une classification et une différenciation significative entre les différents échantillons de farine étudiés sur la base des classes de qualité (les classes E, A et B), la teneur en protéines et le volume du pain.

D'autre part, les autres solutions utilisés ; l'acide lactique et chlorure de sodium ; présentent des réponses significatives de GPT et procurent une bonne classification et une différenciation

entre les différents échantillons de farine sur la base des classes de qualité, la teneur en protéines et la valeur de sédimentation. En présence d'acide lactique, les échantillons de farine ont montré une agrégation rapide du réseau de gluten qui a conduit à un pic fortement défini suivi d'une dégradation rapide. Ces observations sont des caractéristiques des farines de faible qualité technologique (Goldstein et al., 2010). Cette faiblesse est due au faible pH entrainé par l'acide lactique (pH inférieur à 4,0). Ces valeurs inférieures de pH induisent une charge positive importante et une augmentation de la répulsion électrostatique intramoléculaire, entrainant le repliement des protéines et augmentant l'exposition de leurs groupes hydrophobes, en revanche la présence de fortes forces répulsives électrostatiques intermoléculaires empêche la formation de nouvelles liaisons. En outre, à ce faible pH, l'activité protéolytique est encore renforcée et pourrait conduire à la dégradation des protéines du gluten responsables de la fermeté et de l'élasticité de la pâte conduisant à un réseau de gluten mou et moins élastique (Clarke et al., 2004). Par ailleurs, la solution de chlorure de sodium contribue à l'obtention d'une lente formation de réseau de gluten. Cela peut être expliqué par le fait que le sel éloigne les molécules d'eau des protéines du gluten pour interagir avec les ions de sodium et chlorure, induisant une réduction du taux d'hydratation du gluten, et par la suite un prolongement du temps de développement de la pâte, comme il a été démontré dans le travail de McCann et Day (2013). Par conséquent, les résultats obtenus à partir des deux méthodes GPT utilisant l'acide lactique à 5 % et chlorure de sodium à 1 % en tant que solvants ont été utilisés pour calculer les coefficients de corrélation entre les paramètres du GPT et le volume du pain.

En utilisant l’acide lactique, la zone A4-A5 (l'aire sous la courbe de MT à PM) présente la corrélation la plus élevée avec le volume du pain ($r = 0,70$, $p < 0,001$), ce qui laisse supposer que cette zone représente la teneur en gliadine des échantillons de blé. Cela signifie qu'une diminution de la force du gluten causée par une teneur élevée en gliadine nécessite une énergie élevée pour désagréger le réseau de gluten formé, approuvant que la teneur élevée en gliadine soit négativement corrélée avec la force du gluten. Néanmoins, cette corrélation demeure inférieure à celles obtenues entre la valeur de sédimentation, la teneur en protéines et le volume du pain. Cette corrélation s’avère aussi conditionnée par le lieu de croissance puisque différents coefficients de corrélation entre les mêmes paramètres ont été obtenus sur les deux années de récolte 2013 et 2014. Ainsi, GPT utilisant l’acide lactique à 5% ne peut être considéré comme un outil optimal pour remplacer les deux méthodes indirectes : teneur en protéines et valeur de sédimentation dans la prédiction du volume du pain.

Utilisant le chlorure de sodium à 1%, le paramètre AM (le couple 15 s avant le PMT) montre la plus forte corrélation avec le volume du pain ($r = 0,77$, $p < 0,001$, année de récolte 2013 et $r = 0,63$, $p < 0,001$, pour l'année de récolte 2014). Cette observation conduit à supposer que ce paramètre peut refléter la teneur en gluténine des échantillons de blé, puisque cette protéine est capable de générer une structure cohésive du réseau de gluten, en tenant compte qu'une augmentation de la teneur en gluténine augmente la force du gluten et son élasticité (Melnyk et al., 2012). Cette aptitude est justifiée par le fait que les sous-unités à poids moléculaire élevé de la gluténine, qui sont cruciales pour l'élasticité, possèdent un certain nombre de résidus de cystéine disponibles pour former des liaisons intermoléculaires. Par conséquent, un degré de réticulation élevé génère une augmentation de la valeur du paramètre AM et de l'élasticité du réseau de gluten. Cependant, en présence d'une concentration élevée de ce solvant, les forces électrostatiques deviennent négligeables en raison des anions kosmotropes et des cations du sel qui agissent sur la structure de l'eau et, par conséquent, sur l'agrégation du gluten (Zhang et Cremer, 2006). Les Kosmotropes renforcent les interactions hydrophobes entre les protéines du gluten en raison de la disponibilité réduite de l'eau, entraînant une diminution du repliement des protéines et des liaisons hydrogène entre les chaînes non pliées. De ce fait la formation des interactions nécessaires à la conception du réseau du gluten est retardée, comme rapporté par Marti et al. (2015a), qui a aussi utilisé la solution de chlorure de sodium à 0,33 mol / L. Par conséquent, dans notre étude et en raison du faible taux de chlorure de sodium (0,17 mol / L), la formation des interactions protéiques a eu lieu à AM, ce qui explique la bonne corrélation entre ce paramètre et le volume du pain.

Selon les classes de qualité, (E, A, B), l'attribution d'un échantillon donné à une classe de qualité donnée en fonction de la teneur en protéines, la valeur de sédimentation et les paramètres GPT (A4-A5 en utilisant l'acide lactique et AM en utilisant le chlorure de sodium) n'est pas évidente. Cela confirme que la qualité de la farine repose sur plusieurs paramètres physiques, chimiques et rhéologiques, et si pris séparément, ne permettent pas une séparation nette entre les différentes classes (Foca et al., 2007, Marti et al., 2015a, b).

Les corrélations entre la teneur en protéines, la valeur de sédimentation et le volume du pain sont dans la plupart des cas supérieures aux valeurs de corrélation obtenues par GPT. Cependant, la corrélation entre le paramètre AM et le volume du pain est souvent très élevée et stable sur différents lieux de croissance, indiquant que le GPT (avec le chlorure de sodium comme solvant) évalue la qualité des protéines au lieu de mesurer les différences au niveau de la teneur en protéines. Cela signifie que l'interaction génotype-environnement joue un rôle

important dans la capacité du GPT utilisant du chlorure de sodium à 1% comme solvant, à prédire le volume du pain, et le principal facteur influencé par l'environnement est la teneur en protéines. Par conséquent, les résultats de le GPT ne doivent pas être interprétés comme des prédicteurs du volume du pain, à moins que la teneur en protéines ne soit prise en compte.

Selon les résultats obtenus, une combinaison de deux tests rapides est recommandée pour évaluer la qualité boulangère et prédire le volume de pain des échantillons de farine de blé tendre. Par conséquent, parmi les modèles déterminés au moyen d'une analyse de régression multiple, la meilleure prédiction du volume du pain s'avère une fonction linéaire de la teneur en protéines et le paramètre AM. Ce modèle peut expliquer la variation du volume du pain par 63% et fournit des estimations du volume du pain avec une incertitude de ± 39 ml.

La validation de ce modèle a été réalisée au moyen d'une série d'échantillons indépendants (21 variétés différentes de blé panifiable de l'année de récolte 2015 cultivées sur huit sites différents en Allemagne. Les résultats obtenus (64 % de prédictions correctes et 36% de prédictions incorrectes) indiquent que le modèle crée ne devrait pas remplacer la mesure réelle du volume du pain, par le biais des tests de panification, pourtant il pourrait constituer un test de dépistage utile et rapide en étape de sélection du blé pour améliorer la qualité boulangère du blé tendre.

II. Relation entre le macro-polymère de la gluténine et la qualité boulangère du blé tendre sur la base d'une analyse multi-environnementale et multi-génotypique.

Le plus grand des polymères de gluténines est appelé gluténine macropolymère (GMP). GMP est constitué de très grandes structures de HMW et LMW, liées par des liaisons disulfures intra- et intermoléculaires (Don et al., 2003a), avec un poids moléculaire estimé de plusieurs millions de Da ($> 5 \times 10^6$) (Schofield et coll., 1983, Weegels et coll., 1996, Koehler, 2010). Le GMP est insoluble dans la solution de SDS et forme une couche de gel sur le culot d'amidon résultant après centrifugation de la farine de blé tamponnée dans une solution de SDS (Graveland et al., 1979), la raison pour laquelle GMP est aussi connue sous le nom de gel de protéines. Ce GMP peut être isolé en dissolvant les sous-unités de gluténine insolubles dans la solution de SDS sous des conditions réductrices, c'est-à-dire en rompant les liaisons disulfures intermoléculaires, puis peuvent être quantifiées par la méthode HPLC (Cinco-Moroyoqui et MacRitchie, 2008, Cornec et al., 1994).
Des études précédentes ont rapporté le rôle significatif du GMP et de ses sous-unités HMW et LMW ainsi que le rapport LMW / HMW dans l'évaluation de la qualité technologique de la

farine de blé, de sa qualité boulangère et des propriétés de la pâte (Sapirstein et Suchy, 1999 ; Thanhaueser et al. 2014). Moonen et al., 1982, MacRitchie et Lafiandra, 1997 ont démontré aussi une bonne corrélation entre GMP avec d'autres paramètres ; comme la valeur de sédimentation, la force de la pâte, la résistance à l'extension. Cependant seulement quelques-uns des études disponibles ont étudié la relation entre la concentration de GMP et le volume du pain, et ont rapporté qu'ils sont étroitement liés (Thanhaeuser et al., 2014). Pourtant ces précédentes études ont analysé cette relation avec un nombre restreint d'échantillons sans prendre en considération l'impact du génotype et de l'environnement. Cependant, la nature des variétés de blé (génotype) et de l'environnement (lieu de croissance) et leur interaction ont une influence déterminante sur la qualité boulangère du blé, par exemple à travers les variations de la structure du polymère du gluten qui peuvent résulter (Johansson et al. 2001). Donc l'estimation de la corrélation entre le volume du pain et la teneur en GMP des échantillons de blé tendre en intégrant l'influence du lieu de croissance, du génotype et de leur interaction n'est pas encore disponible. C'est pourquoi notre présente étude à investigué un grand nombre d'échantillons de farine de blé tendre provenant de différents sites en Allemagne (huit lieux de croissance de chaque année des deux années de récolte considérées, 2013 et 2014).

Les résultats obtenus ont montré que les échantillons de farine provenant de l'année 2013 présentent des concentrations de GMP plus élevées que les échantillons de farine de 2014, ce qui pourrait s'expliquer par l'effet de chaleur, attribué aux conditions environnementales caractérisant l'année de récolte 2014 en Allemagne (Deutscher Wetterdienst, 2014). Tel que rapporté par Cairns et al., 2012, une température élevée au cours de la phase de remplissage des grains induit des perturbations au moment de la formation des particules du GMP. Ces perturbations sont entrainées comme suit ; le stress thermique accélère les réactions physiologiques et biochimiques dans les grains, ce qui accélère leur taux de déshydratation et réduit par la suite le temps de développement de ces grains. Cette situation ralentit par la suite la biosynthèse de la gluténine, conduisant à une diminution de la concentration de GMP et une augmentation de la taille des particules de gluténine (DuPont et Altenbach, 2003). Ce résultat a été confirmé par les concentrations de GMP de la variété Julius, la seule variété de référence récoltée au cours des deux années de récolte considérées (2013 et 2014). Dans la présente étude, nous avons obtenu une concentration moyenne de 3,9 mg / g en 2013 et une concentration plus faible en 2014 de 3,6 mg / g. La même variété provenant d'un autre lieu de croissance et de l'année de récolte 2010 a été étudié par Thanhaeuser et al. (2014), qui ont

obtenu un rapport LMW / HMW inférieur à celui que nous avons obtenu dans la présente étude.
Cette différence entre nos résultats et les résultats de cette étude peut être l'effet des conditions environnementales (année de récolte et lieu de croissance) ainsi que du dispositif de broyage utilisé, puisque le processus de mouture affecte fortement la composition et la qualité de la farine de blé résultante (El-Porai et al., 2013). Dans notre étude a été utilisé, un broyeur de laboratoire de type Buehler MLU 202 a été utilisé par contre dans l'étude de Thanhaeuser et al. (2014) un broyeur Quadrumat junior de Brabender était choisi.

Ensuite l'effet de la classe de qualité, du génotype et du lieu de croissance sur les paramètres indirects (teneur en protéines et valeur de sédimentation), le volume du pain, les concentrations du GMP et de ses sous-unités a été statistiquement mis en évidence. Pour les échantillons provenant de l'année de récolte 2013, la classe de qualité a été considérée comme une source de variation statistiquement significative pour tous les paramètres considérés ($p \leq 0,001$). Ce résultat est évident puisque le système allemand de classification du blé attribue des échantillons à un certain groupe de qualité sur la base de nombreux caractères de qualité, dont la teneur en protéines, la valeur de sédimentation et le volume du pain, de sorte que le blé de la classe E est caractérisé par la plus haute qualité, suivie du blé de classe A, de classe B et enfin de classe C qui est connu par la qualité la plus basse, et qui n'est pas utilisable pour la panification et donc non étudiée dans notre étude. En outre, la fraction GMP est associée à la teneur en protéines, ce qui explique les résultats obtenus en ce qui concerne les concentrations de GMP, HMW et LMW pour chaque classe de qualité (Spiertz et al., 2006).

Le génotype s'est aussi avéré être une source importante de variation pour tous les paramètres examinés, soit la teneur en protéines, la valeur de sédimentation, le volume du pain, GMP, HMW, LMW et le rapport LMW/HMW. Cependant la distinction entre les différents lieux n'est possible que sur la base de la teneur en protéines, de la valeur de sédimentation et du rapport LMW / HMW. Le lieu de croissance représente la plus grande source de variation de la teneur en protéines ($H = 50,8$, $p \leq 0,001$) par rapport à la classe de qualité et au génotype ($H = 25,7$, $p \leq 0,001$ et $H = 43,9$, $p \leq 0,001$, respectivement). En revanche, le génotype affecte plus les autres paramètres mesurés, ça veut dire la valeur de la sédimentation, le volume du pain, les concentrations de GMP, HMW, LMW et le rapport LMW / HMW. Ce résultat est confirmé par l'étude de Johansson et al. (2001), qui ont signalé que la concentration en protéines est déterminée à la fois génétiquement et environnementalement. Zhu et Khan (2001) ont étudié six génotypes de blé rouge de printemps, cultivés dans sept lieux au Dakota

du Nord en 1998 et ont démontré que GMP était plus contrôlé par les facteurs génétiques qu'environnementaux, ce qui vient d'être confirmé dans notre présente étude.

A l'opposé, les échantillons de blé provenant de l'année 2014 ne présentent aucune différence significative entre les classes de qualité du blé et les génotypes pour GMP, HMW, LMW et le rapport LMW / HMW, ce qui pourrait s'expliquer par l'effet de la chaleur sur GMP et ses sous-unités. Cependant, une différence significative a été démontrée entre les lieus de croissance pour la teneur en protéines, les concentrations en GMP et ses sous-unités, indiquant que le lieu de croissance affecte ces paramètres. Cette différence observée entre les échantillons de blé de 2013 et 2014 peut être attribuée aux températures élevées caractérisant l'année 2014, affectant le grain de blé en termes de divers processus physiologiques et biochimiques, et qui dépend de la réponse et de la sensibilité de chaque génotype à l'environnement (Iqbal et al. 2017, de Leonardis et al., 2015).

Les échantillons de farine de blé provenant de l'année 2013 ont montré une corrélation positive entre les paramètres mesurés (GMP, HMW, LMW), à l'exception du rapport LMW / HMW, et le volume du pain, au contraire des échantillons de blé de 2014 qui n'ont montré aucune relation significative avec le volume du pain. Le coefficient de corrélation le plus élevé obtenu en 2013 a été atteint avec la concentration de HMW ($r = 0,55$, $p < 0,001$). Ce résultat n'a pas confirmé les résultats obtenus par Thanseauser et al. (2014) qui a obtenu une relation étroite entre la teneur en GMP et le volume du pain (GMP : $r = 0,80$, HMW : $r = 0,77$ et LMW : $r = 0,77$). Cette différence pourrait être liée aux différents tests de panification utilisés dans les deux études. Thanseauser et al. (2014) a utilisé le test Micro Rapid Mix comme test de panification avec 10 g de farine et un pétrissage optimisé jusqu'à l'obtention d'une consistance optimale, au lieu du test Rapid Mix qui a été utilisé dans notre étude. Le test Rapid Mix nécessite un pétrissage de 1 minute à une vitesse de 1400 tr / min qui pourrait désintégrer le réseau du GMP, contrairement à un pétrissage doux qui peut maintenir le réseau GMP, dans le cas du test Micro Rapid Mix (1 min à 1250 tr / min). En plus de cela, les différentes méthodes de broyage des échantillons du blé utilisés dans les deux études impactent la teneur en protéines, en GMP et par la suite les différentes valeurs de corrélation. En outre, le coefficient de corrélation entre le volume du pain et les fractions de GMP n'atteint pas les valeurs de corrélation obtenues entre la valeur de sédimentation, la teneur en protéines et le volume du pain.

En ce qui concerne les classes de qualité, le coefficient de corrélation le plus élevé a été attribué aux échantillons de 2013 appartenant à la classe B et obtenu entre le volume du pain et la concentration de HMW, tandis que les échantillons de classe E et A n'ont pas montré de corrélation significative. Ce résultat a démontré que la classe de qualité n'affecte pas significativement la relation entre les fractions GMP et le volume du pain des échantillons de farine de blé tendre. Les échantillons provenant de l'année de récolte 2013 ont montré entre les concentrations de GMP, HMW, LMW et le volume du pian une positive et significative corrélation sur tous les lieux de croissance (sauf le lieu 2) et une corrélation négative entre le volume du pain et le rapport LMW / HMW. Ce résultat est conforme à la conclusion de Wieser et Zimmermann (2000), Jin et al. (2011), Dhaka et Khatkar (2014) qui suggèrent que HMW joue un rôle positif et plus important que celui joué par LMW sur les propriétés rhéologiques de la pâte en contribuant davantage à sa résistance. Les mêmes auteurs ont aussi mis en relief la dépendance de la qualité boulangère du rapport LMW / HMW et rapportent qu'une concentration élevée de HMW par rapport à la concentration de LMW conduit à une meilleure qualité boulangère. De plus, la plus forte corrélation obtenue avec le volume du pain fluctue entre les concentrations de GMP, HMW, LMW et le rapport LMW / HMW. Ces résultats indiquent que la relation entre le volume du pain et ces paramètres dépend de chaque lieu de croissance et du contexte génotypique, confirmant l'étude précédente de Zhu et Khan, (2001) qui supposait que l'environnement et le génotype affectent la formation de GMP, en modifiant la taille moléculaire des polymères de gluténine et en influençant le taux de synthèse des protéines et l'incorporation des sous-unités HMW et LMW.

Néanmoins, presque aucun lieu de croissance ni génotype en 2014 ne représente une corrélation significative entre le volume du pain et les concentrations en GMP et ses sous-unités, qui pourrait s'expliquer par l'effet de la chaleur sur les génotypes présents dans ces lieux, perturbant la biosynthèse des sous-unités GMP, et conduisant à une fausse estimation de la relation entre GMP et le volume du pain.

La nature des variétés de blé a aussi une influence déterminante sur l'évaluation des caractéristiques de qualité, puisque leur degré de sensibilité à l'environnement varie d'une variété à une autre. Par exemple, si une ou deux variétés très sensibles aux changements environnementaux, ou l'inverse, appartiennent à un ensemble d'échantillons avec une sensibilité modérée, pourraient réduire les valeurs de corrélation obtenues et conduire à des conclusions erronées sur l'influence de l'environnement sur les caractéristiques de la qualité du blé (Lukov & McVetty, 1991). En plus, les concentrations de GMP, HMW et LMW ne

sont pas les seuls facteurs affectant la relation avec le volume du pain, la composition de HMW affecte aussi la concentration de GMP et le volume du pain (Shewry et al., 1992).

III. Prédiction de l'effet de mélange et de la qualité boulangère des mélanges de blé tendre au moyen d'Extensographe, de GlutoPeak®-Test et du macro-polymère de gluten dans des conditions de fertilisation et d'environnement variables.

Outre l'évaluation de la qualité boulangère des échantillons purs de farine de blé (précédemment étudiée), la prédiction de la qualité boulangère des mélanges de farine de blé tendre était également une question d'intérêt de la présente thèse.

L'investigation de la qualité boulangère des mélanges de farine ne peut être réalisée sans penser à l'effet de mélange. L'effet de mélange est un effet synergique, observé chez les mélanges de farines et qui se traduit par l'obtention d'un volume de pain supérieur ou inférieur au volume de pain mathématiquement calculé à partir des volumes de pain des farines de blé mélangées. Par conséquent, avant l'évaluation de la qualité boulangère et la prédiction du volume de pain, l'évaluation de la valeur de l'effet de mélange (BEV) a été réalisée en tenant compte de l'impact du génotype, du lieu de croissance et du niveau de la fertilisation azotée. Un avantage majeur pourrait être tiré de cet effet de mélange, qui est une amélioration du volume du pain tout en réduisant le niveau de la fertilisation azotée à des fins économiques et écologiques.

Quelques études antérieures se sont occupées de la clarification des causes de l'effet de mélange et ses influences sur les propriétés de la pâte et les performances en termes de panification. Bushuk et al. (1983) a supposé que cet effet pourrait être le résultat des effets synergiques causés par les différentes qualités protéiques des farines de blé mélangées, et ne dépend pas de leur teneur en protéines. Tandis que Bolling (1980, 1983) a rapporté que l'effet de mélange et les propriétés rhéologiques de la pâte sont étroitement liés, indiquant que la complémentarité entre les variétés de blé est atteinte. Ainsi, Schäfer et Flechsig (1985) ont démontré que les variétés de blé à pâte molle sont renforcées par des variétés de blé ayant de fortes propriétés de pâte, ce qui augmente le volume de pain des mélanges de blé. En outre, Schäfer et Flechsig (1985) rapportent aussi que l'effet de mélange est plus important lorsque la résistance à l'étirement et les propriétés d'élasticité caractérisant chaque variété intégrée dans le mélange sont différentes. En conséquence, la relation entre les propriétés d'agrégation du gluten mesurées par le biais du GPT, les propriétés rhéologiques de la pâte déterminées au moyen d'Extensograph, la concentration de GMP et de ses sous-unités, la valeur de l'effet de

mélange et le volume de pain des mélanges de blé a été étudiée, soulignant l'effet du génotype, de l'environnement (lieu de croissance) et le niveau de fertilisation (faible, moyen, élevé).

Selon les résultats obtenus, BEV qui correspond à la différence entre le volume du pain du mélange observé (obtenu par le test de panification : RMT) et le volume du pain calculé (obtenu à partir des valeurs du volume du pain des échantillons de farine pure intégrés dans ce mélange) peut être positif, négatif ou insignifiant (BEV inférieur à ± 15 ml / 100g de farine). D'après les résultats obtenus dans la présente étude, il semble que le lieu de croissance, le génotype, le niveau de fertilisation et leur interaction ont une grande influence sur la BEV. Cependant, il semble que le niveau élevé de fertilisation azotée n'a pas d'impact sur la BEV, puisqu'elle atteint les valeurs les plus élevées à un niveau de fertilisation moyen. Cette observation semble être confirmée par la stabilité de la BEV du mélange Memory - Julius avec les niveaux de fertilisation moyenne et élevé dans le lieu 1 (42 ml / 100g et 41 ml / 100g, respectivement), mais cette stabilité pourrait aussi être le résultat de la complémentarité entre les deux variétés intégrées dans le mélange (Memory et Julius), et qui s'avèrent comme les variétés aux propriétés de pâte les plus complémentaires.

Néanmoins, l'analyse ANOVA a confirmé qu'une fertilisation azotée élevée n'appliquait pas automatiquement une BEV positive et élevée, car un niveau de fertilisation moyen peut conduire au même effet de mélange obtenu avec un niveau élevé. Cette BEV ne semble pas être influencée par les interactions génotype × niveau de fertilisation et génotype × lieu de croissance, mais s'est révélée conditionnée par l'interaction lieu de croissance × niveau de fertilisation, ce qui signifie que l'ampleur d'une significative différence entre les différents niveaux de fertilisation dépend du lieu d'où les partenaires du mélange composant les mélanges proviennent. Mais cette interaction n'a été observée qu'à certains lieux entre le niveau faible et élevé de fertilisation et élevé et entre le niveau faible et moyen, tandis que pour l'interaction entre le niveau moyen et élevé, aucune différence significative n'a été obtenue à travers tous les lieux de croissance. Ces résultats confirment notre observation précédente et approuvent qu'une fertilisation azotée réduite du niveau élevé au moyen n'ait pas d'impact sur la BEV et que le même résultat peut être obtenu avec un niveau de fertilisation moyen ou élevé. Notre résultat ne peut pas être comparé avec d'autres études, parce que l'effet du génotype, du lieu et du niveau de fertilisation sur l'effet de mélange n'a pas été auparavant étudié, et doit être davantage élucidé.

Les paramètres du GPT, les concentrations de GMP et de ses sous-unités et les paramètres d'Extensograph ont été étudiés pour évaluer et prédire la BEV et le volume du pain. Puisque la prédiction de l'effet de mélange n'a pas été étudiée précédemment, il n'y a pas encore de méthode spécifique pour prédire la BEV et le volume du pain des mélanges, la raison pour laquelle deux méthodes ont été appliquées. Ces méthodes sont basées sur les caractéristiques de qualité des partenaires du mélange, à savoir les paramètres du GPT, les concentrations de GMP et de ses sous-unités et les paramètres d'Extensograph des deux échantillons de farine de blé pur impliqués dans chaque mélange.

La première méthode est basée sur les valeurs moyennes des paramètres considérés des deux échantillons de farine formant le mélange (méthode 1 :$\bar{X}$), tandis que la seconde méthode implique la différence absolue entre les résultats des deux échantillons de farine formant le mélange (méthode 2: Δ). La deuxième méthode a été prise en considération vu que l'étude de Bolling (1968), a suggéré que la différence entre les propriétés rhéologiques de la pâte des échantillons de farine formant le mélange et la BEV de ce mélange sont liés de manière linéaire. Le résultat de la corrélation entre la BEV et les paramètres étudiés a démontré que la corrélation la plus élevée est obtenue entre BEV et ΔHMW ($r = 0,65$, $p < 0,001$), (aussi avec $GMP_{\bar{X}}$: $r = 0,59$, $p < 0,001$) et est seulement atteinte à un niveau élevé de fertilisation. Ces corrélations augmentent du faible niveau de fertilisation au niveau élevé (sauf $HMW_{\bar{X}}$ et ΔGMP au niveau moyen), de ce fait il semble que le niveau de fertilisation joue un rôle important la relation entre BEV et la concentration en GMP et ses sous-unités. Ceci peut s'expliquer par l'impact important de la fertilisation azotée sur les concentrations en GMP et HMW, de sorte que l'azote renforce la synthèse de HMW et augmente sa concentration, qui est étroitement corrélée avec la concentration en GMP comme rapporté par Yue et al. (2007).

Pourtant la relation entre les paramètres d'Extensograph, calculés selon la méthode 2, et BEV demeure faible, ce qui contredit le résultat obtenu par Bolling (1984b), qui a suggéré qu'une différence élevée entre les valeurs d'extensibilité des partenaires de mélange conduit à un effet de mélange élevé. La différence entre notre résultat et les résultats de cette étude peut être due à la présence de seulement 18 échantillons de mélanges de farine qui montrent une BEV positive de parmi les 135 échantillons de mélanges de farine testés. De plus, Bolling (1968) n'a pas étudié des échantillons de mélanges présentant une BEV négative, ce qui est le cas dans notre étude.

En revanche, parmi les paramètres étudiés, le paramètre $R_{\bar{X}}$ (45min) (résistance à l'extension mesurée après 45 min de fermentation) calculé selon la méthode 1 montre la corrélation la plus élevée et la plus significative avec le volume du pain de tous les mélanges (r = 0.66, p <0.001), au contraire des paramètres du GPT qui ne présentent aucune relation significative avec le volume du pain. Cette relation reste significative sur tous les niveaux de fertilisation (r = 0,67, p <0,001 au niveau faible, r = 0,75, p <0,001 au niveau moyen et r = 0,74, p <0,001 au niveau élevé), mais demeure plus stable contre l'augmentation de la fertilisation azotée du niveau moyen au niveau élevé, plutôt que du faible au moyen.

Le rapport $(R/E)_{\bar{X}}$ mesuré à 45 min montre une bonne corrélation avec le volume du pain sur les trois niveaux de fertilisation (r = 0,57, p <0,001 au niveau faible, r = 0,73, p <0,001 au niveau moyen et r = 0,68, p <0,001 au niveau élevé). Ces résultats sont en accord avec les résultats de Ktenioudaki et al. (2011), qui a démontré une relation significative entre le volume du pain et les paramètres rhéologiques de la pâte. $GMP_{\bar{X}}$ et $LMW_{\bar{X}}$ ont aussi montré une corrélation significative avec le volume du pain au faible niveau de la fertilisation azotée (r = 0,62, p <0,001 et r = 0,64, p <0,001, respectivement), mais cette relation devient non-significative avec l'augmentation du niveau de fertilisation azotée, contrairement aux résultats de Yue et al. (2007). Cela peut être dû à l'impact du lieu de croissance, de génotype et leur interaction avec le niveau de fertilisation azotée, tel que rapporté par Triboi et al. (2000), et qui dépasse l'effet de l'augmentation du niveau de fertilisation sur GMP et ses sous-unités. De plus, la taille des particules des sous-unités de GMP est également un facteur important dans la détermination de la qualité boulangère du blé, et l'effet du niveau de fertilisation sur celui-ci peut également influencer notre résultat.

Puisque la relation entre BEV et les paramètres étudiés est restée non significative, une analyse de régression sur les meilleurs sous-ensemble a été réalisée avec BEV de tous les mélanges des trois niveaux de fertilisation comme variable dépendante et la teneur en protéines, valeur de sédimentation, concentrations de GMP et de ses sous-unités et tous les paramètres d'Extensograph mesurés au trois temps de fermentation (45min, 90 min et 135 min) ont été inclus dans l'analyse en tant que variables indépendantes, afin de développer un modèle permettant une meilleure prédiction de BEV. Cependant, tous les modèles produits n'ont pas fourni d'explication significative de la variation du BEV. Cela signifie que les paramètres Extensograph (En, R, E, et le rapport R / E), même en association avec les autres paramètres considérés ne sont pas de bons prédicteurs pour les mélanges de farine provenant

de différents niveaux de fertilisation. La relation entre ces paramètres et le BEV reste dépendante du niveau de fertilisation, du génotype, du lieu et de leurs interactions.

Mais d'autre part, le paramètre $R_{\bar{X}}$ (45 min) en association avec la teneur en protéines et la valeur de sédimentation s'avèrent être de bons prédicteurs du volume de pain des mélanges de farine (R2 = 0,69). Cela signifie que le modèle créé au moyen des paramètres mentionnés peut fournir une explication de la variation du volume du pain avec un pourcentage de 69% et une incertitude de ± 23 ml. Ensuite, une validation croisée a été effectuée pour tester si les résultats obtenus peuvent s'adapter à d'autres échantillons. Il en résulte une bonne corrélation entre le volume de pain observé et prédit sur les trois niveaux de fertilisation ($r = 0,89$, $p < 0,001$ au niveau faible, $r = 0,84$, $p < 0,001$ au niveau moyen et $r = 0,83$, $p < 0,001$ au niveau élevé). Ces résultats indiquent que le modèle créé impliquant les valeurs moyennes des paramètres suivants : teneur en protéines, valeur de sédimentation, concentration de GMP et le paramètre R (45 min) des échantillons partenaires intégrés au mélange, peut être utile pour expliquer la variation du volume de pain des mélanges quel que soit le niveau de fertilisation azotée (faible, moyen ou élevé), et prédire par la suite la BEV qui n'est normalement observé qu'au moyen des tests de panification.

Chapitre 4 : Conclusions générales

Le travail actuel a été structuré en trois parties principales; Premièrement, la prédiction de la qualité boulangère du blé tendre à l'aide d'une méthode optimisée du GlutoPeak®-Test, suivie de l'analyse de la relation entre GMP et la qualité boulangère du blé tendre et enfin la prédiction de l'effet de mélange et de la qualité boulangère des mélanges de blé tendre au moyen d'Extensographe, de GPT et de GMP dans des conditions de fertilisation, d'environnement et de contexte génotypique variables.

Pour traiter le premier lot de travail, 15 variétés différentes de blé panifiable de l'année de récolte 2013 et 11 variétés de l'année de récolte 2014, cultivées dans huit lieux différents en Allemagne ($n = 120$, 2013 ; $n = 88$, 2014) ont été analysées au moyen du GPT après optimisation de cette méthode, en utilisant différents solvants (eau distillée, 5% de saccharose, 5% de carbonate de sodium, 5% d'acide lactique et 1% de chlorure de sodium) et en faisant varier le rapport farine / solvant. Des corrélations ont été établies entre la teneur en protéines brutes, la valeur de sédimentation, les paramètres d'agrégation du gluten mesurés au moyen du GPT et le volume du pain. Enfin, une analyse de régression multiple a été réalisée pour mettre en évidence la relation entre le volume du pain et divers tests de dépistage. Le modèle

le mieux adapté a été validé par une série d'échantillons indépendants de l'année de récolte 2015.

Les résultats de cette partie ont montré que parmi les solvants utilisés pour exécuter le GPT, la solution de chlorure de sodium à 1% était la plus appropriée pour obtenir une réponse stable et significative par rapport au volume du pain. Une fonction linéaire de la teneur en protéines et du paramètre AM s'est révélée utile pour expliquer la variation du volume du pain dans différents lieux de croissance. L'évaluation de cette fonction linéaire avec un ensemble de données indépendant conduit à 64% de prédictions correctes et à 36% incorrectes. Cela indique que la stratégie consistant à associer deux tests rapides est efficace dans une certaine mesure et que l'application du modèle peut être utile pour la sélection de variétés dans l'objectif d'améliorer la qualité boulangère du blé tendre. Pourtant cette méthode ne peut pas remplacer la mesure réelle du volume du pain par un test de panification standard.

Dans le deuxième lot de travail, les GMP de 15 variétés de blé tendre d'hiver 2013 et 11 variétés de blé tendre de l'année 2014, cultivées sur huit sites différents en Allemagne, ont été extraits dans des conditions réductrices et quantifiées par la méthode HPLC. L'impact du génotype et le lieu de croissance sur le volume du pain, la concentration en GMP et ses sous-unités (GMP, HMW, LMW), le rapport LMW / HMW et sur leur corrélation avec le volume du pain ont été démontrés.

Les résultats obtenus ont démontré que les concentrations en GMP et ses sous-unités des échantillons de farine de blé de l'année de récolte 2013 étaient plus influencées par des facteurs génétiques qu'environnementaux, contrairement aux échantillons de farine de blé de l'année de récolte 2014, qui mettent en relief l'impact du génotype sur les fractions de GMP. La relation entre le volume du pain et les concentrations en GMP et ses sous-unités des échantillons de l'année 2013 s'avèrent dans certains cas significatifs et dans d'autres cas non significatif car il reste sous l'influence du contexte génétique des échantillons et du lieu de croissance. Cette relation est révélée non significative pour les échantillons de farine de blé provenant de l'année 2014, en raison de l'effet de chaleur, caractérisant cette année, et qui peut conduire à une diminution de la formation du GMP. Ceci indique que les concentrations de GMP et de ses sous-unités ne peuvent pas être une approche utile pour remplacer le test de panification et prédire le volume du pain sans tenir en compte l'effet de l'environnement et du génotype. Une étude de la composition des sous-unités du GMP pourrait également être d'une

grande envergure pour mettre en évidence le processus impliqué dans cette relation avec le volume du pain.

Enfin, dans le troisième lot de travail, six variétés de blé tendre différentes de l'année de récolte 2015 ont été cultivées en trois endroits différents en Allemagne et avec trois niveaux différents de fertilisation azotée (faible, moyen et élevé). Ces échantillons de farine étaient mélangés à un rapport de 50 : 50, et ont été testé dans cette étude, pour mettre en évidence l'effet de mélange, sa relation avec différents paramètres de qualité et l'impact du génotype, du lieu de croissance et du niveau de fertilisation sur cette relation et sur la variation de la valeur de l'effet de mélange (BEV). Les propriétés d'agrégation du gluten des échantillons de farine pure et de mélange ont été analysés au moyen du GPT, les propriétés rhéologiques de la pâte au moyen de l'Extensographe, tandis que les GMP ont été extraites dans des conditions réductrices et quantifiées par la méthode HPLC.

Les résultas obtenus ont indiqué que l'effet de mélange qui peut être positif, négatif ou insignifiant est influencé par le génotype, le lieu de croissance, le niveau de fertilisation et l'interaction lieu de croissance × niveau de fertilisation. Cependant, aucune différence significative n'a été obtenue entre les niveaux de fertilisation élevé et moyen sur les sites considérés, ce qui implique que la fertilisation tardive avec un niveau élevé d'apport d'azote n'affecte pas la BEV et que la réduction de la fertilisation azotée du niveau élevé au niveau moyen est possible. Cependant, le niveau de fertilisation reste une source cruciale de variation de la relation entre les paramètres GPT, GMP et Extensograph calculée sur la base des caractéristiques de qualité des échantillons partenaires intégrés dans le mélange de farine et la BEV ce qui rend sa prédiction au moyen de ces paramètres, même en association impossible. Parmi ces paramètres, la résistance à l'extension (R) mesurée à 45min de fermentation a montré la corrélation la plus élevée, significative et stable avec le volume du pain du mélange. Une fonction linéaire de R (45 min), GMP, teneur en protéines et valeur de sédimentation peut fournir une explication de la variation de 69% du volume du pain avec une incertitude de ± 23 ml, quel que soit le niveau de fertilisation azotée. D'autres études sont nécessaires pour renforcer ces résultats et étudier l'effet de différents rapports de mélange sur la BEV, sur la base d'une analyse pluriannuelle, -génotype et –environnemental (lieu de croissance) dans le but de tirer profit de l'effet de mélange, considéré comme une nouvelle approche pour augmenter la qualité boulangère des variétés de blé tendre et réduire le niveau de fertilisation azotée.

Chapter 1

Introduction

General introduction

Wheat (*Triticum aestivum* L.) is throughout history one of our most important food crops and contributed approximately 29.15% of the 2600 million tons of cereal grains produced in the world in 2016 (FAO 2017). This importance of wheat is due to several reasons: it is cultivated in nearly all regions of the world. Thus, it represents the main source of income for millions of small scale farmers living in developing countries around the globe. Moreover, wheat can be stored for long periods of time, to produce different types of wheat-based foods, supplying an affordable source of nutrition to a large portion of the global population. Those described qualities have made wheat an important food crops for food security, and the most traded cereal crop in the international agricultural food market, impacting the economy of farmers and other food-chain sectors in countries around the world.

In addition to that, due to the presence of gluten proteins in wheat flour, main source of the viscoelastic properties of wheat flour dough, the greatest proportion of wheat flour produced is used for bread making. As a consequence, wheat baking quality assessment remains a great challenge on the different levels of wheat bread production. In early stages, breeders need to discern if the wheat varieties they breed and grow will fulfil the attributes required by the processing industries and consumers. Moreover, cereal traders and millers are also permanently facing the challenge to select the right wheat quality necessary for their specific interest, while bakers need to determine the quality of the milling products they ordered to meet customers' requirements and expectations relative to the baked products (Lindhauer, 2014). Hence, many efforts have been made and a great attention was paid over years to study the baking quality of wheat and to predict it. Since many decades scientists have established a system of methods suitable to evaluate wheat baking performances. As direct methods, baking tests are still considered the most accurate way to assess wheat baking quality, and the volume of the loaf is generally taken as the single factor on which judgement is based. However, baking tests necessitate specific equipment of baking facilities, experienced staff, time and a considerable quantity of wheat material, which is an inappropriate condition in early stages of breeding. Moreover, the variability that can occurs during the baking test execution, and the dough over- or under-kneading can generate differences in the baking quality assessment (Canja et al, 2014).

Therefore, a broad range of indirect methods were established to characterise the wheat varieties properties, and subsequently assess the baking quality performances. The grain

protein content, as indirect method, is globally still the main criterion in evaluating wheat baking quality, as it showed a linear relationship with the loaf volume, and the gluten proteins are recognized as the most important components governing bread making quality (Wrigley and Bietz, 1988). Nevertheless, in recent years some approved wheat varieties in Germany do not have a linear dependency between protein quantity and loaf volume. Those varieties have relatively low grain proteins contents; however, because of the high functionality of the gluten proteins, the baking performance of such wheat varieties is much better than expected from classical quality parameters (Lindhauer, 2014). These observations highlight the fundamental impact of gluten proteins quality on dough forming and baking quality. As a result, in most recent years the classical quality parameters appear to be no longer suitable to assess the baking quality of modern German wheat varieties (Seling, 2010; Kazman 2010).

According to the described situation, a reliable assessment of baking quality of soft wheat flour samples by means of new method is nowadays of major importance for breeders and millers, as well as for bakers. Consequently, current studies focus on investigating new methods to assess loaf volume and baking quality, on the basis of gluten performances.

Among these methods, GlutoPeak®-Test (GPT) has been proposed for the quick evaluation of wheat baking quality by measuring the gluten aggregation properties (Kaur and Seetharaman, 2011), while other studies focused on gluten macro polymer (GMP) content, which is the major part of glutenin, with the highest molecular weight, and unextractable in sodium dodecyl sulphate solution (Don et al., 2003a; Graveland et al., 1982; Weegels et al., 1996). Both methods require very small amount of wheat material, which would be a characteristic of high interest to breeders in early stages of breeding.

In spite of that, only a few of the available studies enquired into the relationship between loaf volume and GPT parameters, or GMP content (Thanhaeuser et al., 2014; Weegels et al., 1996).In addition to that, the available scientific studies tested the GPT method or investigated the GMP content with a restricted number of samples, which could not highlight the impact of genotype, environment and their interaction on the applicability and the ability of the tested methods to assess the wheat baking quality. However, genotype, environment and their interaction influence the various components of flour protein, thus environmental factors might modify molecular sizes of glutenin polymers by influencing the rate of protein synthesis and incorporation of high molecular weight (HMW) and low molecular weight

(LMW) subunits in forming glutenin polymers which are highly associated with the baking performances (Johansson et al., 1999; Zhu and Khan, 2001).

Furthermore, by wheat flour blends, there is not yet a scientific knowledge about the prediction of the blending effect value (BEV), resulting from the blending effect which is a noticeable synergistic effect by flour blends, and demonstrated as a higher or lower loaf volume, as mathematically expected. This blending effect can be clarified only through a detailed understanding of the influence of genotype, location of growth and fertilisation level, which is also not yet clear.

Thereby, the aim of this study is to determine new quality parameters based on gluten aggregation properties measured by the GPT, and on the GMP subunits concentrations in order to assess the baking quality and to predict the loaf volume of soft wheat flour samples. This determination is carried out on the basis of a multilocation and multigenotype analysis, in order to highlight the impact of genotype and location of growth on the ability of these methods to predict the baking performances of soft wheat flour samples.

In addition to dough rheological properties of wheat flour blend samples determined by means of the Extensograph, the previous two methods (GPT and GMP subunits) are also applied in order to assess the blending effect of soft wheat flour blends and afterwards take advantage of it for the purpose of predicting loaf volume of blend samples, which will be of economic benefit to farmers and help to reduce environmental effects associated with nitrogen fertilisation.

To achieve these aims, the GPT method was optimized varying the solvent/flour ratio and using different solvents as alternative to pure water in order to investigate a set of different winter wheat varieties harvested at 8 different locations in Germany over two years. Afterwards, the crucial fraction of the glutenins which is isolated from wheat flour as a SDS insoluble gel layer (GMP) of flours from two set of different winter wheat varieties harvested at 8 different locations in Germany from harvest year 2013 and 2014 was extracted under reducing conditions and quantified using HPLC. Finally, a set of six soft wheat varieties, harvested at three locations in Germany and with three levels of nitrogen fertilisation were blended with 50% to 50% level of mixture. In addition to GMP extraction and quantification by means of the HPLC, the gluten aggregation properties and the dough rheological properties of the pure and the blend soft wheat flour samples were determined by means of GPT and Extensograph, respectively.

Accordingly, the thesis is organised in three main parts:

1. Prediction of bread wheat baking quality using an optimized GlutoPeak®-Test method.
2. Relationship between glutenin macro polymer and bread wheat baking quality on the basis of multilocation and multigenotype analysis.
3. Prediction of blending effect and baking quality of soft wheat blends by means of Extensograph, GlutoPeak®-Test and glutenin macro polymer under variable fertilisation levels and environmental conditions.

1. Wheat

The agricultural literature features various works on the origins of wheat. The most credible assumption is that people began to use wheat for food in prehistoric times, beginning at least 15,000 years B.C.

Wheat as we know it in the millennia of this era is not the same as it was at the very beginning. Through natural crossings, mutations, and natural selection these have evolved into all the many varieties of wheat grown worldwide. The genetics of wheat show that its development is very complex (Symko, 1999). Wheat belongs to the genus Triticum and is a polyploid species, a common outcome of plant evolution. The evolution of wheat was a consequence of series of natural hybridization events that eventually led to hexaploid (genomes A, B and D) wheat. This evolution goes through the various forms of cultivated wheat: a diploid; *Triticum monococcum* (einkorn), tetraploids; *Tritium turgidum, Triticum dicoccum* (emmer) and *Triticum durum* (durum), and hexaploids; *Triticum spelta* and *Triticum aestivum* (spelt and common wheat also known as bread wheat, respectively) (Gustafson et al., 2009; McIntosh, 2004). Einkorn and emmer are still cultivated and now mentioned as ancient grains. Durum is used primarily for pasta production, while bread wheat which is the most widely cultivated wheat today, is used to make the wide variety of dough and batter-based products. *Triticum aestivum* is made of three genomes pairs (AA, BB, and DD) of each of the seven basic chromosomes (42 chromosomes total).

Bread wheat arose by two separate hybridizations. The first hybridization has been occurred between two diploid Triticum and Aegilops species, which are *Triticum urartu* (AA) and *Aegilops speltoides* (BB) and resulted in a tetraploid species; *Triticum turgidum* (AABB). The second hybridization occurred between this new cultivated tetraploid species and onother diploid species; the wild grass *Triticum tauschii* (also called *Aegilops tauschii* and *Aegilops squarosa*) and leading to the hexaploid species *Triticum aestivum* (AABBDD) and *Triticum spelta* (AABBDD). The A and B genomes of the generated tetraploid and hexaploid species are respectively contributed by *Triticum urartu* and *Aegilops speltoides*, while the D genome of hexaploid speciest is clearly derived from that of *Triticum tauschii* (Wang et al., 1997; Snape and Pankova, 2006).

The evolution of bread wheat is illustrated in Figure 1, which also shows examples of spikes and grain.

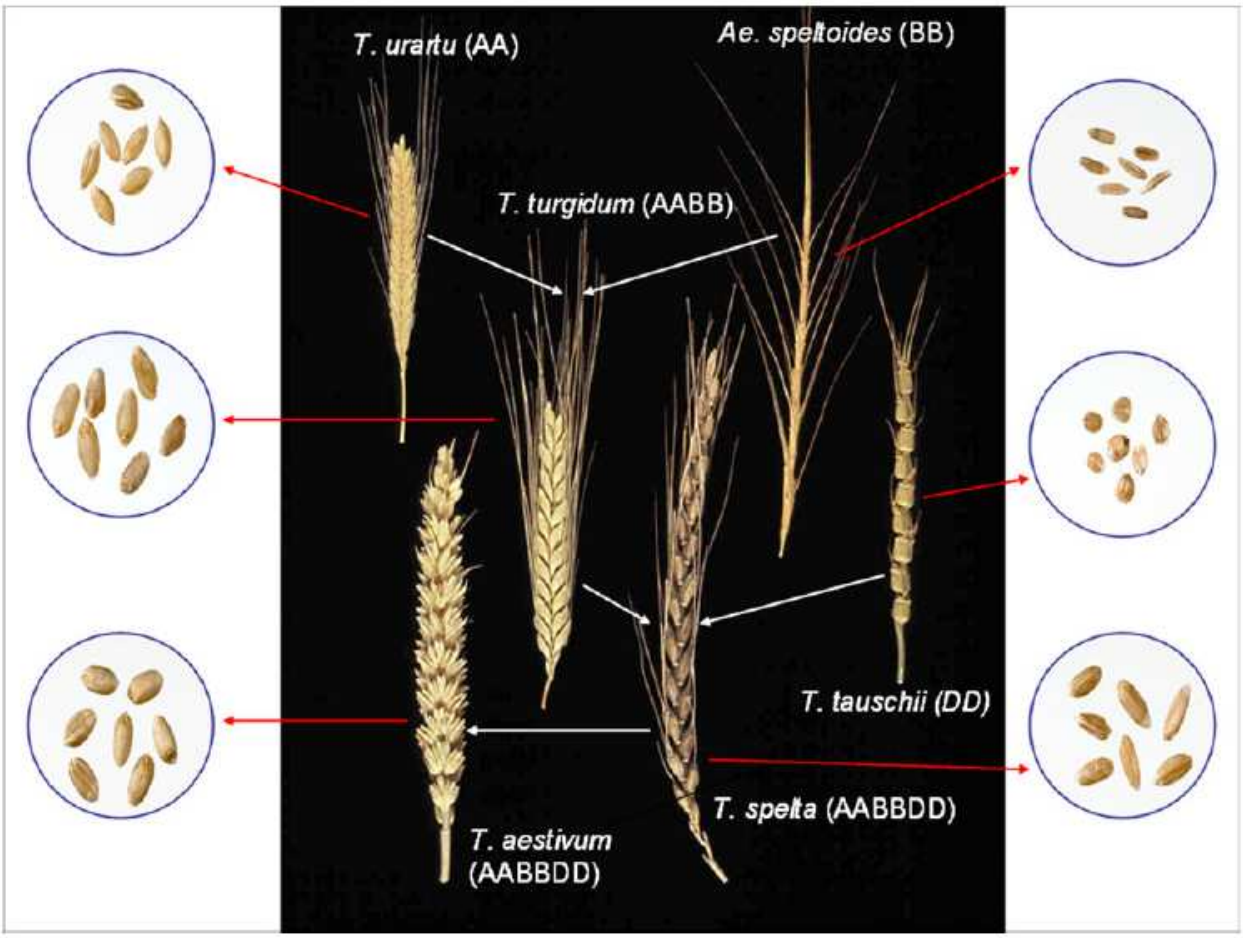


Figure 1: The evolutionary and genome relationships between Triticum aestivum (commen wheat), Triticum spelta (durum wheat) and wild diploid grasses specie, showing examples of spikes and grains (Snape and Pankova, 2006).

Wheat belongs to a large classification of cereals, which also includes maize (*Zea mays* L.), rice (*Oryza spp*.), barley (*Hordeum vulgare* L.), sorghum (*Sorgum spp*.), millet (*Pennisetum glaucum* R.Br.), oats (*Avena sativa* L.), and rye (*Secale cereale* L.). Of the 2600 million tons of cereal grains produced in the world in 2016, wheat contributed approximately 29.15 % to this total (FAO 2017). Throughout history wheat has been an important component of the humankind diet due to its agronomic adaptability, ease of storage, nutritional goodness, and the ability of its flour to produce a variety of palatable, interesting, and satisfying foods. Wheat also gained importance because of its free-threshing trait that made the grain easy to harvest.

1.1. Wheat classification

Three categories of traits related to the physical hardness of the kernel, the seed coat colour and the growing season are often used to distinguish and describe bread wheat types.

For the physical kernel hardness, bread wheat is classified as hard or soft wheat. This hardness is related to the energy required to mill wheat grains into flour. Hard wheat milling necessitates high energy and more force to crush it, in comparison with soft wheat (Atwell, 2001; Feiz et al., 2008). Based on seed coat colour, wheat is classified as red and white wheat. This colour depends on the presence or absence of a reddish pigment in the outer layers of the wheat kernel, which can be revealed with a visual examination (Atwell, 2001; Bechtel et al., 2009). And finally, based on growing season, two wheat types are distinguished: winter and spring wheat. Winter wheat is planted in the autumn, sprouts in the spring and known by its low temperatures requirement to permit vernalization. This process usually occurs at temperatures < 8° C and is demanded for winter wheat to ensure proper flowering time and successful seed reproduction, that means this period is required before heads formation, that contain the wheat kernels. If winter wheat is not vernalized, the plant will grow but never produce heads with new seeds (Atwell, 2001; Amasino, 2004; Miglietta et al., 1995). Spring wheat is planted in spring and doesn't need vernalization for heads formation. Both, winter and spring wheat are harvested in summer, while spring wheat can also be harvested in autumn. Consequently, *Triticum aestivum* forms the classes hard red winter wheat, hard red spring wheat, hard white winter wheat, soft red winter wheat, hard white wheat and soft white wheat (Atwell, 2001).

In addition to *Triticum aestivum*, two other species are also grown (*Triticum compactum* and *Triticum durum*). *Triticum compactum*, called club wheats are usually characterized by low protein content and are always soft. Moreover, white and red varieties, at the same time as winter and spring club wheat varieties exist. Because club wheats are grown in small quantities, it is often included in soft white bread wheat class. *Triticum durum*, which includes red durum and durum wheat classes, is identified by much harder kernels than common hard wheat, and doesn't require the process of vernalization (Atwell, 2001).

1.2. Wheat kernel anatomy

The wheat kernel is a complex structure, composed of several components with specific compositions and structures, as illustrated in Figure 2. These sections can be divided into three main anatomical regions, called bran, endosperm and germ (Atwell, 2001).

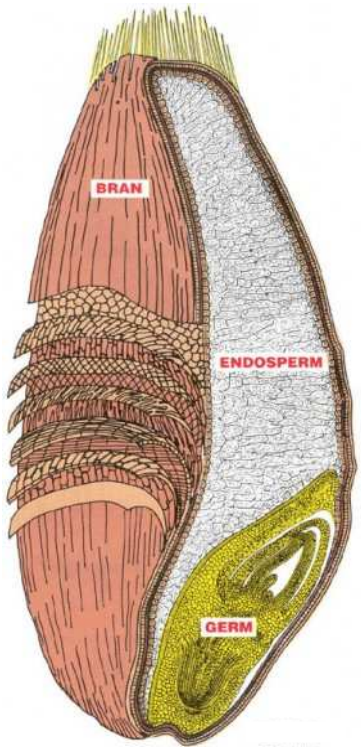


Figure 2: Illustration of the wheat kernel anatomy, showing the germ, the endosperm and the bran made up of many different layers (courtesy wheat food council).

Bran is the outer tough layer of the wheat kernel, which protects the grain and constitutes about 14 % of the total grain weight. Bran is made of two principal components: pericarp and seed coat (Atwell, 2001; Bechtel et al., 2009; Delcour and Hoseney, 2010). The pericarp is the outer layer of the wheat kernel, composed of epidermis, subepidermal layer, parenchyma, cross cells and tube cells. The pericarp constitutes the ripened ovary wall, which is dead at harvest ripeness. The seed coat has also a crucial impact on the wheat kernel so that it controls the water transfer between the seed and the surrounding area (Evers and Bechtel 1988).

The next wheat kernel constituent is the endosperm, which represents the major component of the grain (80 to 85 % of its weight) and the primary constituent of flour. This component is located in the centre of the gain and formed by cells, in which the starch particles are

implanted in a protein matrix (Delcour and Hoseney 2010; Evers and Millar, 2002). The endosperm provides the energy and protein for the developing wheat plant. A unique layer formed by endosperm cells is located between the bran and the starchy endosperm. This layer called aleurone layer is mostly removed during flour milling operations due to its location in the wheat kernel (Atwell, 2001; Bechtel et al., 2009; Delcour and Hoseney, 2010).

Finally, the germ represents about 3 % of the kernel weight, and contains most of the essential nutrients for the kernel (Atwell, 2001). The germ is formed by two main components: the embryonic axis and the scutellum. The embryonic axis represents the rudimentary root and shoot, while the scutellum is a storage organ and transports nutrients from the endosperm to the germ during germination (Bechtel et al., 2009; Evers and Bechtel, 1988; Posner and Hibbs 1997).

1.3. Composition of wheat kernel

The wheat kernel is mainly composed of carbohydrates, principally starch accounting for approximately 60 % to 70 % of the total dry matter (DM) of the grain, and non-starch polysaccharides, known as dietary fibre (≈ 10 % DM of the grain). The second important group of constituents are the proteins which fall within an average range of about 9 – 15 % DM of the grain. The remaining portions of the wheat kernel consist of moisture, lipids and finally as minor constituents, vitamins and minerals (Koehler and Wieser, 2013; Shewry et al, 2010).

Starch is the fundamental food reserve in wheat kernel and represents the main component with unique physical, chemical, and nutritional properties in the carbohydrates fraction of the grain. Starch occurs only in endosperm in the form of granules formed in amyloplasts. Each amyloplast contains one granule that can be either large or small. Large granules are lenticular with a diameter of 25 to 40 µm, while small granules are spherical with a diameter of 5 to 10 µm (Delcour and Hoseney, 2010; Stone and Morell, 2009).These granules are made up of the two water-insoluble glucose polymers amylose (20–30 %, approximately) and amylopectin (70–80 %) (Konik-Rose et al., 2007). Amylose is characterized by a linear chain of glucopyranose units connected through α-D-(1-4) glycosidic linkages and is almost linear with a degree of polymerisation in the range of 500–6000 glucose residues. Nowadays, it is well recognized that a fraction of the amylose constituting molecules also have α-(1-6) -linkages, giving this part a slightly branched structure (Hizukuri et al., 1981; Shibanuma et al., 1994). In contrast, amylopectin is a very large highly branched polysaccharide, with a degree

of polymerisation ranging from 3×10^5 to 3×10^6 glucose units, providing a considerably higher molecular weight (10^7 - 10^9) than amylose. It is composed of chain of glucopyranose units connected by α-D-(1-4)-glycosidic linkages which are interlinked by a α-(1-6)-bonds (Buléon et al., 1998; Zobel, 1988). Consequently, amylopectin has greater solubility and solution stability than amylose due to its extensive branching (Lineback and Rasper 1988). Moreover, amylose is characterized by an amorphous form in the wheat kernel, differently from amylopectin which includes semicrystalline lamellae (BeMiller and Huber, 2008).

In addition to starch, other types of carbohydrates are present in the wheat kernel and are primarily constituents of the cell walls, known as non-starch carbohydrates, and from nutritional point of view dietary fibre. The main types of non-starch polysaccharides are arabinoxylans, cellulose, β-glucans and arabinogalactans (Delcour and Hoseney, 2010).

The arabinoxylans are the predominant non-starch polysaccharides in wheat grain, and the major fraction (85 – 90 %) of the so called pentosans (Delcour and Hoseney, 2010; Koehler and Wieser, 2013). The arabinoxylans backbone chain is made up of β-(1-4)-linked D-xylopyranosyl units, with arabinose substitutions to which ferulic acid can be covalently linked via ester bonds (Meuser and Suckow, 1986). Based on solubility, arabinoxylans can be subdivided into two categories: water extractable arabinoxylans (WEAX) and water unextractable arabinoxylans (WUAX) (Delcour and Hoseney, 2010; Saulnier et al., 2007). It is reported that the mean reason of the insolubility of WUAX in water is the high crosslinking between arabinoxylans molecules (Delcour and Hoseney, 2010; Stone and Morell, 2009).

Besides arabinoxylans the penstosan contains also another water extractable fraction, which is the arabinogalactan peptide. It is a highly branched fraction, consisting of β-(1-3) and β-(1-6) linked galactopyranose units with α- glycosidically bound arabinofuranose residues (Kohler and Wieser, 2013).

Cellulose is also a linear polymer made up of several β-(1-4) linked glucopyranosyl units, forming polysaccharide chains. The chains are joined together in a three-dimensional structure and stabilised through intermolecular hydrogen bonds and van der Waals interactions. This structure maintains the cellulose molecule in an inflexible conformation and makes it very insoluble and resistant to hydrolysis (Delcour and Hoseney, 2010; Stone and Morell, 2009).

Finally, as non-starch carbohydrates, (1-3) -(1-4)-β-glucans referred to as *β*-glucans made up of a linear chain of D-glucose linked with β-(1-4) and β-(1-3) glycosidic bonds (Woodward et al., 1983; Li et al., 2006), and show a higher water solubility than arabinoxylans und cellulose (Stone and Morell, 2009).

In wheat, lipids are minor constituents, representing approximately 3 % of total grain weight and they are mainly stored in the germ, to a smaller extent in the aleurone layer and to the lesser extent in the endosperm (Koehler and Wieser, 2013). The lipids are present as either starch lipids (linked to starch) or non-starch lipids. Non-starch lipids that make up approximately 75 % of the total flour lipids are classified as free, extractable in petroleum or diethyl ether, or bound, extractable only in cold polar solvent mixtures (MacMurray and Morrison, 1970). Free lipids can further be classified into a polar (glycolipids and phospholipids) and non-polar lipid fraction (mainly triglycerides). Bound lipids are consisting of a part of the phospholipids and glycolipids that can be associated, for example with proteins (Delcour and Hoseney, 2010). Starch lipids are classified as non-polar and polar also (Delcour and Hoseney, 2010; Wrigley et al., 2009). The major non-polar lipids, triglycerides, are the dominating lipid class in the germ and the aleurone layer, while phospho- and glycolipids are present in the endosperm (Hoseney, 1994).
The mineral content of wheat grain ranges from approximately 1.0 to 2.5 %, and varies significantly according to wheat variety, growing and soil conditions and fertilisation (Koehler and Wieser, 2013; Bock, 2000). The major constituents of the mineral fraction of wheat are magnesium, phosphorus and potassium, with significant amounts of iron, zinc, manganese and copper (Davis et al., 1984). The major portion of the minerals (>90 %) is in the bran, the aleurone layer and the germ (Koehler and Wieser, 2013), therefore the milling process can result in significant losses of minerals.

Wheat grain contains vitamins in concentrations ranging from below 1 to approximately 50 mg/kg (Koehler and Wieser, 2013). Wheat grain is a good source of B-vitamins (thiamine, riboflavin, niacin and pyridoxine), as well as fat-soluble vitamins (tocopherols). Like minerals, these vitamins are concentrated in particular in the aleurone layer and in the germ, thus milling process of wheat grains reduce their levels or remove most of the vitamins.

Despite the fact that, wheat is known primarily to be rich in carbohydrates, wheat also has the highest protein content of the cereal grains. The protein fraction is known to have the most essential role for bread making (Morrison, 1988; Wall, 1979). As reported by previous

studies, baking quality depends on the presence of specific proteins and protein subunits, as well as the molecular weight distribution of polymeric proteins (Morrison, 1988; Wall, 1979; Johansson *et al.*, 1993, 2001; Gupta *et al.*, 1993).

2. Wheat proteins

2.1.Classification and characteristics of wheat proteins

The average protein content of wheat grains may vary from 9 % to more than 15 % DM (Koehler and Wieser, 2013). This content varies according to the genotype, the growing conditions, and particularly the amount and time of nitrogen fertilisation. Proteins are distributed over the whole wheat grain with different concentrations; however, the starchy endosperm includes the highest protein concentration (≈ 13 %) (Belitz et al, 2009), therefore it is considered the main source of white flour obtained by milling the wheat gains and sieving.

According to their solubility in water, salt solution, aqueous alcohols and dilute acids or bases, proteins have been classified by Osborne (Osborne, 1907) into four fractions: albumins, globulins, gliadins and glutenins. Albumins are soluble in water, whereas globulins are soluble in dilute salt solutions and insoluble in pure water. Gliadins classically known to be soluble in aqueous alcohols, like 60–70 % ethanol, and glutenins in dilute acids or bases. However, the Osborne classification excluded a notable glutenins fraction, which is insoluble in dilute acids and its extraction by means of strong bases solutions destroys its primary structure (Koehler and Wieser, 2013). Furthermore, the Osborne classification is defined to be strongly influenced by experimental conditions and cannot provide a clear separation of wheat proteins on the basis of the biochemical and functional characteristics. Nevertheless, this fractionation method is still often very useful as an initial separation step to obtain semi-pure protein fractions (Goesaert et al., 2005).

Nowadays, wheat proteins are also classified into non-gluten proteins (functional proteins) and gluten proteins (Shewry, 2003) as summirized in Table 1. The non-gluten proteins consist mainly of albumins and globulins and cover between 15 to 20 % of the total wheat grain proteins (Goesaert et al., 2005; Delcour et al., 2012). They are in the aleurone layer, bran, and germ, whereas their concentration in the starchy endosperm is relatively low (Belderok et al., 2000). Most of the non-gluten proteins are metabolic proteins, for example enzymes or enzyme inhibitors (Koehler and Wieser, 2013; Šramková et al., 2009). Gluten proteins (between 80 and 85 % of total wheat protein) are collectively known as storage proteins because they supply the seedling with nitrogen and amino acids during germination (Shewry

et al., 1995; Shewry and Halford, 2002). Gluten proteins consist of gliadins and glutenins and vary in molecular size, molecular weight, solubility, and functionality. They are present in the endosperm of the mature wheat grain where they form a continuous matrix around the starch granules. In the presence of hydration and under mechanical conditions, gliadins and glutenins form a viscoelastic three-dimensional network material called gluten, which represent the rubbery mass that is left when wheat flour is washed with water to remove starch, non-starch polysaccharides, and water-soluble constituents. Gluten confers properties of elasticity and extensibility that are essential for functionality of wheat flours (Shewry *et al.*, 1995). These properties make wheat unique among cereals, with the ability to be transformed into great diversity of food products, breads, noodles, pasta, cookies, pastries and many other foods (Day et al., 2006).

Table 1: Classification of bread wheat proteins (non-gluten and gluten proteins), their subunits and molecular weight.

	Group	**Subunits**	**Structure**	**Molecular weight (Da)**
Non-gluten proteins	Albumins	-	Monomeric	< 25 000
	Globulins	-	Monomeric	
Gluten proteins	Gliadins	α - Gliadins β - Gliadins γ - Gliadins ω - Gliadins	Monomeric	30 000 - 75 000
	Glutenins	HMW subunits LMW subunits	Polymeric	80 000 – 120 000 30 000 – 55 000
	Gluten macro polymer	HMW subunits LMW subunits	Polymeric	> 5×10^6

2.1.1. Albumins and globulins

The water-soluble albumins and salt-soluble globulins have lower molecular weights (MW) than 25 000 Da (Veraverbeke and Delcour, 2002). Alpha- amylase/trypsin, serpins and purothionins are the predominant albumins and globulins in wheat grain (Østergaard *et al.*, 2000; Garcia-Olmedo *et al.*, 2002). Generally, albumins and globulins are not thought to play a critical role in flour quality, although, minor importance on bread-making quality has been

reported (Schofield and Booth, 1983), however they are considered to have nutritionally better amino acid compositions because of their higher lysine and methionine contents as compared to the rest of the proteins in the wheat grain (Lasztity, 1984). These non-gluten proteins help as inhibitors of insects and fungal pathogens in protecting embryo before germination (Dupont and Altenbach, 2003).

2.1.2. Gliadins

Gliadins are monomeric proteins that consist of single chain polypeptides with molecular weights ranging between 30 000 to 75 000 Da (D'Ovidio and Masci, 2004; Goesaert et al., 2005; Delcour et al., 2012). They represent about 40 to 50 % of the total protein content in wheat and are soluble in 70 % (v/v) ethanol (Qi et al., 2006; Thewissen et al., 2011). Biochemically, gliadins are classified into four sub-types based on their molecular mobility at low pH in acid polyacrylamide gel electrophoresis and amino acid compositions: alpha- (α-), beta- (β-), gamma- (γ-), and omega- (ω-) gliadins (Shewry et al., 1986). The α- and β-gliadins molecular weights are similar thereby they are often referred to as α-type gliadins, and consequently many authors classified gliadins into 3 groups only: α-, γ-, and ω- types (Kuktaité, 2004). Gliadins are rich in proline and glutamine and contain a high number of cysteine residues (Shewry, 2003); therefore, they were accordingly classified as 'Sulphur (S)-rich proteins'. Cysteines have tremendous influence on the functional properties of wheat proteins since they represent the most chemically reactive group found in proteins (de Jongh, 2007). Most α- type gliadins contain six cysteine residues and γ-gliadins eight cysteine residues located in positions able to form intra-chain cysteine disulphide bonds. While the ω-gliadins, which lack cysteine residues ('S-poor proteins') cannot form disulphide bonds (Delcour et al., 2012; Lafiandra and MacRitchie, 1997; Wieser, 2007). Subsequently, ω-gliadins have relatively expanded structures, in comparison with α- and γ-gliadins configurations (Lafiandra and MacRitchie, 1997). The α- and γ- gliadins types are the major components of wheat varieties, whereas the ω-gliadins occur in much lower proportions (Wieser and Kieffer, 2001). Due to the described situation, no free and reactive sulphide bonds are available to form intermolecular cross-links (Lindsay and Skerritt, 1999).

In dough formation, the gliadins cannot become covalently-linked into large elastic networks as the glutenins, but they act as a 'plasticiser' and contribute mainly to the viscosity and extensibility of dough, which are important rheological characteristics of dough. Gliadins may interact through hydrophobic interactions and hydrogen bonds and associate with other gliadins or glutenins (Veraverbeke and Delcour, 2002).

2.1.3. Glutenins

Glutenins consist of high molecular weight-glutenin subunits (HMW-GS), with around 10 % of the total gluten proteins content and the predominant low molecular weight-glutenin subunits (LMW-GS) with approximately 20% of the total gluten proteins content. The apparent molecular weight based on SDS-PAGE of HMW and LMW fall in the range of 80 000 - 120 000 and 30 000 - 55 000, respectively (Lafiandra and MacRitchie, 1997; Southan and MacRitchie, 1999; Delcour et al., 2012). These two fractions are obtained after reduction of the glutenins by means of a disulphide reducing agent such as dithiothreitol (DTT) (Delcour et al., 2012; Lafiandra and MacRitchie, 1997). Furthermore, after reduction and according to their mobility profile on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) the HMW-GS and LMW-GS can be classified in four groups (A-B-C-D regions). The A region correspond to the HMW-GS, while the B, C and D regions refer to LMW-glutenins (Delcour et al., 2012; Wrigley, 1996; Gianibelli et al., 2001). The amino acid sequences of LMW-GS in C and D regions are similar to amino acid sequence of (α; γ), and ω gliadins, respectively (Gianibelli et al., 2001). According to Delcour et al, 2012, the main difference among B, C and D regions is the first amino acid residue that forms their structure, which can be serine, methionine, or isoleucine.

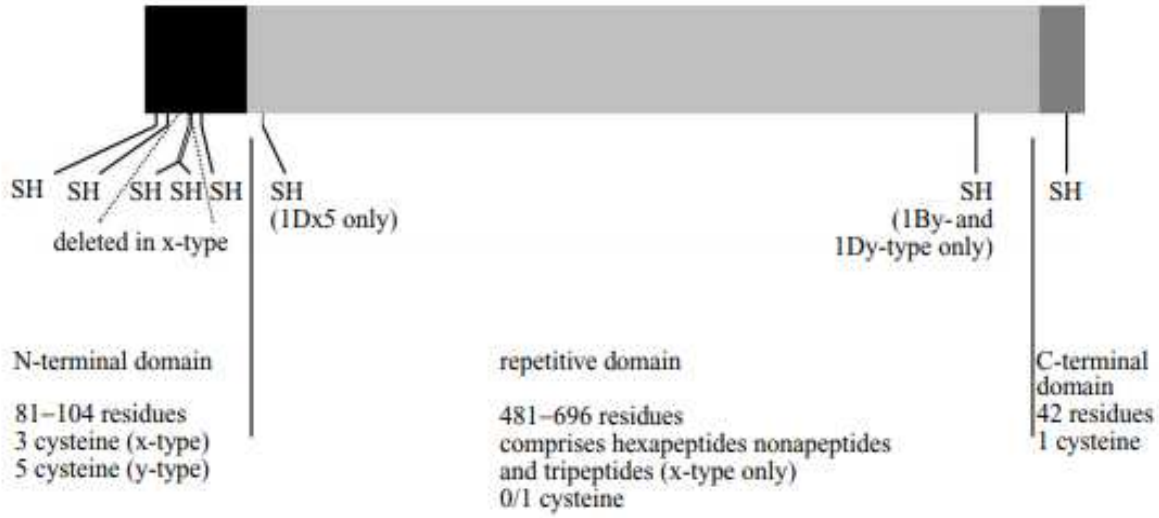


Figure 3: Schematic summary of the three domains (N-terminal domain, repetitive domain and C-terminal domain) of the amino-acid sequences of x-type and y-type HMW subunits (Shewry et al, 2002).

The single loci encoding HMW subunits are present on the long arms of the group 1 chromosomes (1A, 1B, 1D), and each locus comprising two genes encoding subunits that differ in their properties and are called x-type and y-type subunits (Payne 1987). A number of genes encoding HMW subunits have been isolated from bread wheat and it was shown that the amino-acid sequences comprise three distinct domains (Shewry et al., 1992; Reddy and Appels 1993). The central domains of the HMW subunits consist of repeated peptides, based on two or three short peptide motifs. They vary in length from about 420 to 700 residues and account for between 74 and 84% of the whole HMW subunit. These domains are flanked by short non-repetitive domains, which vary in length from 81 to 104 residues at the N-terminus but always comprise 42 residues at the C-terminus (Figure 3). The HMW-GS x-type and y-type have three and five cysteine residues, respectively. Therefore, intermolecular disulphide cross-links can be formed, which is underlined by the fact that HMW-GS do not appear as monomers in bread wheat dough (Shewry et al., 1992) and which enables HMW-GS and LMW-GS to be linked via of intra- and intermolecular disulphide bonds forming polymeric networks with molecular weights of several millions (Lafiandra and MacRitchie, 1997).

Although HMW-GS account no more than 10 % of total flour protein (Payne, 1986; Wieser, 2007); they are the most important determinants of bread-making quality because of their importance in forming the glutenin polymers (Shewry et al., 1992).

As a result of this polymerizing capacity, the molecular weight of glutenin can vary over wide limits. At the lower limit, this can be roughly 100 000. The upper limits have been difficult to determine, but values in the order of millions with a wide distribution have been obtained (Schofield et al 1983).

The largest glutenins polymer is named glutenin macropolymers (GMP). GMP consists of very large structures of both HMW and LMW, linked by intramolecular and intermolecular disulfide bonds (Don et al., 2003a), with a molecular weight up to multimillions Da estimated to $> 5 \times 10^6$, indicating that it may belong to the largest proteins in nature (Schofield et al 1983; Weegels et al., 1996; Koehler 2010). GMP is insoluble in sodium dodecyl sulphate (SDS) solution and forms a gel layer on top of the starch pellet after centrifugation of wheat flour buffered in SDS solution (Graveland et al., 1979), therefore it's known as gel protein (Figure 4). GMP can be isolated by dissolving the SDS unextractable glutenin subfractions under reducing conditions (i.e, interchain disulfide bonds are broken) and subsequently, quantitated using gel permeation HPLC (Cinco-Moroyoqui and MacRitchie, 2008; Cornec et al., 1994). Previous studies reported the significant functionality of GMP and its HMW and LMW subunits as well as the ratio LMW / HMW in assessing wheat flour quality, baking

quality, and dough properties (Sapirstein and Suchy, 1999; Shewry, 2009; Thanhaueser et al., 2014).



Figure 4: GMP gel obtained after extraction with 1.5 % SDS and centrifugation (80 000 × g, 20 °C for 30 min) before (left) and after (right) separation from the starch pellet (Mueller, 2018).

2.2.Impact of genetic background and environmental conditions on wheat proteins

Many studies substantiated the effect of the genetic background and the environmental conditions, mainly temperature regimes and water availability characterizing the location of growth as well as nitrogen fertilisation and their interactions on wheat proteins.

According to Weegels et al., 1996; Johansson and Svensson, 1998, genetic background influences strongly the wheat protein composition and content, which in turn impacts dough characteristics and bread making quality. Variation in amount and size distribution of wheat proteins according to genotype might be due to the differences in structure, number of cysteine residues and hydrophobicity between different protein subunits (Lindsay and Skerritt, 1998; Shewry et al., 1992). This statement was confirmed by the studies of Shewry et al., 1992; Johansson et al., 1999, who highlighted that the presence of the allele 1Dx5 of HMW-GS leads to better bread making quality, due to its composition rich in cysteine residues in comparison with the allele 1Dx2, which provides an increased polymer size.

Environmental conditions characterizing location of growth have also a significant influence on the concentration of specific proteins, protein subunits as well as polymeric proteins size distribution (Johansson et al., 2001, 2002; Dupont and Altenbach, 2003).

Previous studies reported that heat stress, defined as where temperatures are hot enough for sufficient time that they cause irreversible damage to plant function or development (Hall, 2001) and drought stress, occurring when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation (Jaleel et al., 2009), mainly affected the quantity and quality of grain protein through the shortening of grain-filling period. According to Blumenthal et al. (1995), heat stress (> 35 °C) induces an increase of the gliadins/glutenins ratio and a decrease of the proportion of large polymers in wheat flour. However, heat stress effect remains dependant on genotype, thus genotypes having alleles for the HMW-GS 1Dx5, 1Dy10 generally showed more stability in response to high temperature than that with 1Dx2, 1Dy12 HMW-GS alleles (Blumenthal, 1995; Panozzo and Eagles, 2000; Irmak et al., 2008). On the other hand, after anthesis stage drought stress reduced the period of grain filling but didn't influence accumulation rates of different protein fractions (Daniel and Triboi, 2002).

Nitrogen fertilisation plays a crucial role during wheat growing, either for gain yield or protein synthesis. Normally wheat nitrogen fertilisation is split into three applications at the beginning of the vegetation wheat growth stages to ensure an adequate grain yield, followed usually by late-season nitrogen fertilisation in order to increase grain protein content (Olson and Kurtz, 1982). As previously demonstrated, nitrogen fertilisation doesn't influence only grain protein quantity (Fuertes-Mendizabal et al., 2010; Garrido-Lestache et al., 2005; Wieser and Seilmeier, 1998), but also protein quality through its composition (Johansson et al., 2001). With the increase of fertilisation level, the quantities of gluten proteins increase, however the effects on gliadins are more pronounced than on glutenins resulting in an elevated gliadin/glutenin ratio (Martre et al., 2006). Particularly according to the studies of Wieser and Seilmeier, 1998, ω-gliadins and HMW-GS proportions increased, while LMW-GS and γ-gliadins proportions decreased by high nitrogen fertilization and the amounts of albumin and globulin remain unaffected. However, the effect of this late nitrogen fertilisation varies noticeably according to the conditions of growth location, harvest year, timing of fertilisation and type of nitrogen fertilizer (Fuertes-Mendizabal et al., 2010, 2013; Garrido-Lestache et al., 2004, 2005).

3. Milling and bread making

3.1. Milling process

The milling process is the grinding of the wheat kernels to smaller particles that can be transformed into desirable and palatable food products (Atwell, 2001). Nowadays, modern milling processes enable a physical separation of the three anatomical structures of the wheat kernel (bran, endosperm, and germ) followed by the reduction of the endosperm particles into flour (Atwell, 2001). For milling, three general operations are involved: cleaning, tempering, and milling.

Cleaning is a necessary step to remove all non-wheat kernel components, termed “dockage” that consist of other types of seeds, damaged wheat kernels, insects, stems, stones and other debris and hazardous material, which could reduce flour quality or damage the mill (Atwell, 2001). This operation is usually based on separation of debris by size, shape, density and magnetism.

After cleaning, predetermined amounts of water are added to temper wheat kernels, followed by a time interval before milling. According to Fang and Campbell (2003), the purpose of this procedure, termed tempering is to toughen the bran, making it more flexible to be separated from the endosperm and germ, resulting in reduced formation of small bran particles amounts that could contaminate the flour. Moreover, it softens the endosperm, facilitating its grinding into flour with less force. The amount of water added depends on the initial moisture content and the hardness of the wheat kernel, while tempering time rages between 8 and 24 hours (Posner and Hibbs, 1997). Generally soft wheat requires less water and less time than hard wheat. As reported by Glenn et al., 1991, tempering as a compromise between maintaining optimal dryness for an easy sifting and an adequate moisture to toughen the bran and soften the endosperm, induces strength and stiffness decrease, but increases elasticity and plasticity characteristics.

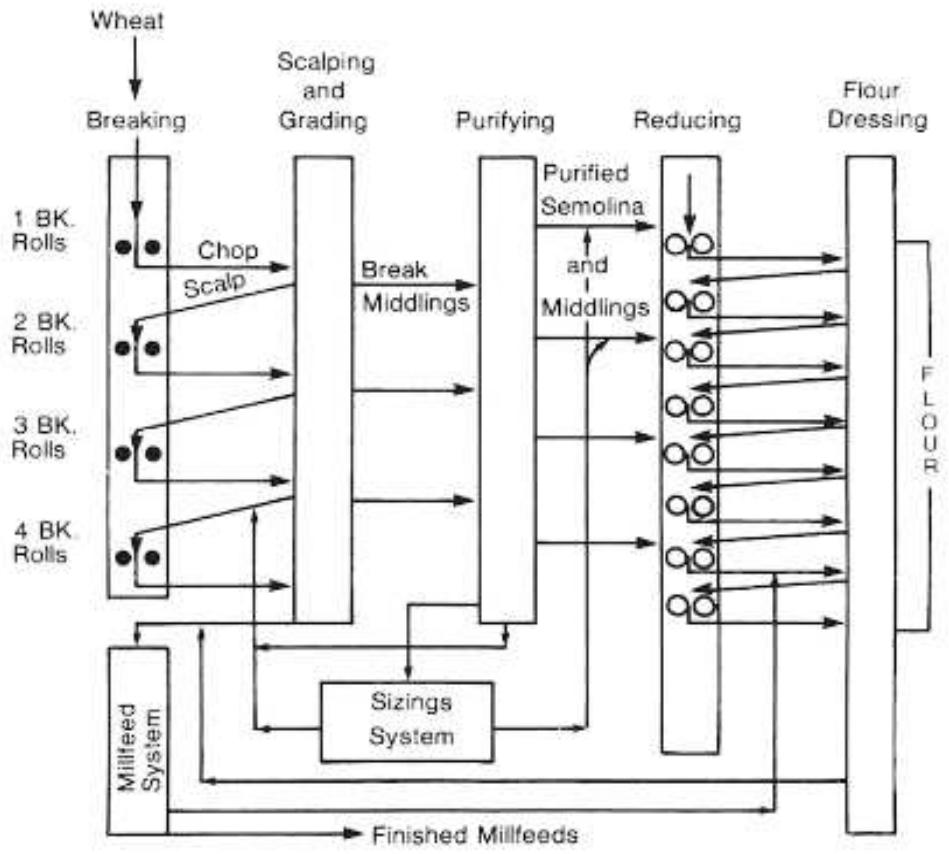


Figure 5: Simplified flow diagram of flour milling showing the major processing systems (break, reduction, sizing, flour dressing and millfeed systems) (Atwell, 2001).

After tempering, the wheat is ready for milling and is run through the mill. The milling process involves two principal grinding systems: break and reduction separated by sizing, flour dressing and millfeed systems (Figure 5). The break system is designed to remove the endosperm from the bran and germ, by means of break rolls possessing spiral grooves, called corrugations, turning at different speeds in opposite directions (Figure 6). Generally, there are about four or five roller mills in the break system as shown in figure 5.

The germ and the bran are removed in the break system, thus the germ is compressed when it goes through the rolls, while the bran particles are usually in the form of low density small flakes, which facilitate the separation from the endosperm fraction shattered into particles. After each break and by means of sieves and / or purifiers the ground material is separated on the basis of size and density (sizing system). The small particles are conducted to the flour, and the large particles are either removed like the germ and the bran or sent to the next break system. From the break system emanate middlings (large particles of endosperm) and break flour. The middlings are subsequently transferred to the reduction system to reduce the size

particles (lower than 150 µm) producing flour and remove the final particles of fine bran. The rolls of the reduction system are smooth in comparison with rolls of break system (Atwell, 2001; Hsieh et al., 1980). The large percentage of the particles composing the final flour comes off the reduction system.

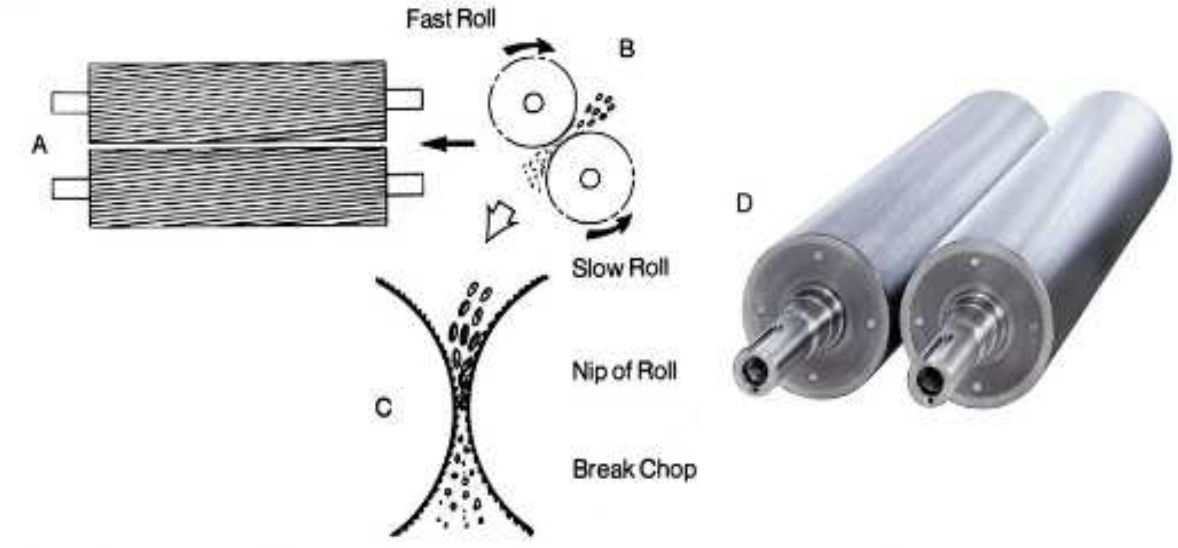


Figure 6: Break rolls of the break system shown in longitudinal (A) and cross (B) sections, possessing corrugations (A and B). The rolls milling action is shown in B and C (Atwell, 2001).

Flours obtained from break and reduction system can be combined in many ways to create different types of flour, but it is usually sifted again in the flour dressing system. In this process, small particles that pass through the sieves meets the particle standard size of the flour and the large particles are recirculated bach to the appropriate point in the grinding process. Finally, the germ and the bran are separated from each other in the millfeed system and the adhering endosperm is channelled into the flour dressing system. The coarse bran resulting from the first steps of grinding is called bran (about 11 % of the total mill products), while the finer branny material resulting from the later steps is called shorts (about 15 %) (Atwell, 2001). The bran, shorts and germ products are usually sold separately in order to be used as animal feed, or to be integrated in food products as ingredients for human consumption.

In mainland Europe, flours are primarily classified according to the ash content which is related to the extraction rate of wheat flour that means the level of bran contained in flour.

Table 2: Equivalent of German wheat flour types in France, Italy and USA

Country	Germany	France	Italy	USA
Wheat flour type	Type 405	45	00	Pastry flour
	Type 550	55	0	All-purpose flour
	Type 812	80	1	High gluten flour
	Type 1050	110	2	First clear flour
	Type 1600	150	Farina integrale	Whole wheat flour

In Germany, wheat flour types relate to average ash % × 1000. Apart from whole wheat, which includes the entire fractions of the wheat kernel (extraction rate equal to 100 %), five types of wheat flour are known in Germany: Type 405 is made from soft wheat, with approximately an extraction rate up to 55 – 66 % and an ash content < 0.50 % DM. This type is known as pastry flour and used to make baked goods, such as muffins, biscuits, cookies, etc. Type 550 is equivalent to all-purpose flour, with an ash content varying from 0.51 % DM to 0.63 % DM. It is made from a blend of soft and hard wheat with an extraction rate of 65 – 70 %. This flour type is mainly used for breads, cookies, biscuits, muffins, etc. While flour type 812, termed bread flour (extraction rate 75 – 80 %) is the best type to give bread the needed structure to rise and hold its shape. It's made from hard wheat and has an ash content varying from 0.64 % DM to 0.90 % DM. Flour type 1050 (extraction rate 80 – 85 %), also known as high gluten flour, is characterized by a high ash content ranging from 1.21 to 1.80 % DM. This flour type is made from hard wheat and used in conjunction with other grains and flour to provide more structure. Finally flour type 1600, is equivalent to whole wheat flour and derived from the whole wheat kernel, including the germ and bran, with an extraction rate ranging from 90 to 95 %. This type has an ash content of approximately 2 % DM (Manley, 2011). The closest French, Italian and American equivalents of the German wheat flour types are mentioned in Table 2.

3.2. Bread making and baking reactions

The bread making process comprises three stages: ingredients mixing, fermentation periods alternated with punching operations of the dough, in order to remove or reduce the occluded gas in the dough and bring the ingredients back into a cohesive structure; and finally baking.

During those three stages dough is subjected to different shear and large extensional deformations, which are largely affected by temperature and water hydration, as well as to several physical and chemical changes (Rosell and Collar, 2009).

The basic ingredients of bread baking are: flour as the major structural element, water, yeast and sodium chloride. In addition to its ability to produce gas by converting carbohydrates into carbon dioxide and ethanol, yeast has a crucial effect on dough rheological properties (Mirsaeedghazi et al., 2008). Sodium chloride, commonly known as salt has also major functions; it improves the sensory attributes of bread and has a strengthening effect on the gluten during dough mixing (Niman, 1981). It was also reported that salt is used to overcome the low pH of dough since dough with low pH requires a shorter mixing time and have less stability than dough with normal pH level (Hoseney, 1985, 1994). Two optional ingredients can be added in dough for bread making: sugar as a source of fermentable carbohydrates for the yeast and malt flour, which is rich in diastase, in order to compensate for the low diastatic activity of flour. The diastatic activity is a measure of the ability of flour to produce maltose, which is a needed sugar for yeast fermentation from its starch by the action of its own amylase (Nechita et al., 2009). Other ingredients can also be added to the dough to obtain satisfactory product. Fat or emulsifiers are often added as dough strengtheners and crumb softeners. Flour improvers, such as ascorbic acid, as often used to improve dough strength, resulting in bread with a higher loaf volume and a better crumb texture (Belderok et al., 2000).

As first stage in bread making, the basic and auxiliary ingredients are mixed to obtain a homogeneous mixture, as well as dough with visco-elastic properties. Moreover, during mixing a quantity of air is included in dough, which is crucial for rheological dough properties and for the quality of the final product (Canja et al., 2014). The dough has to be mixed for an optimum time to fully develop, and at this stage it offers maximum resistance to extension. Undermixing may cause small unmixed patches that interfere in the proofing stage. While, excessive mixing changes dough properties from good (smooth and elastic) to poor (slack and sticky), and induces a decrease in the consistency, which is attributed to the weakening of the protein network (Sliwinski et al., 2004).

When the ingredients are thoroughly mixed, the tough will be submitted to the first fermentation. During fermentation, the yeast breaks carbohydrates down into carbon dioxide and alcohol. This reaction is accelerated by the enzymes present in yeast and flour. The carbon dioxide produced causes the dough to rise through the growth of air bubbles

previously incorporated during mixing, while the alcohol produced mostly evaporates from the dough during the baking process. During the first fermentation, dough becomes more flexible and elastic while developing the gas retaining properties. These properties are required to produce bread with optimal loaf volume (Belderok et al., 2000).

After the first fermentation, the dough is divided into pieces and rounded by hand or machine. During this punching operation, most of the carbon dioxide formed in the first fermentation will be removed. This loss of gas will be compensated in the following fermentation stage.

Afterwards, the fermented dough pieces are moulded in order to give them the appropriate shape. This operation varies according to the bread type desired. In the case of tin bread, the moulding consists of sheeting, rolling the dough sheet into a cylinder which is put into pan. Whereas in the case of bread rolls, the dough pieces are divided, rolled into balls and placed on a metal plate, which after a final fermentation, will be baked.

Finally, after the final fermentation the dough portions are baked in oven for a certain time, depending on the size of the dough pieces (Belderok et al., 2000).

The growth of gas bubbles during proof and baking determines the characteristics of the bread structure and thus the ultimate volume and texture of the baked product (Rosell, 2011).

During bread making, dough undergoes several changes due to different baking reactions. At the beginning of the baking stage, there is a strong evaporation of moisture from the exterior layers of the dough. This evaporation is moderated by means of the condensation of moisture onto the dough piece surface, which retains consequently a degree of elasticity. At the same time, the increase of the internal temperature of the dough induces intense fermentation stage with carbon dioxide production, resulting in an important size development of dough pieces.

Heating in the presence of water induces also a disruption of the molecular structure of starch granules. This process is called gelatinisation and is a unique property of starch (Atwell et al., 1988; BeMiller and Huber, 2008). Commonly, at room temperature and during mixing, starch absorbs water up to 50 % of its DM leading to a starch suspension. This process is increased with temperature that means more water permeates into the starch granules as the temperature increases. The primary consequence of the gelatinisation process is the loss of internal molecular order and crystallinity, characterising amylopectin granules (Tester and Debon, 2000; Waigh et al., 2000). Subsequently, amylose dissolves in water (amylose leaching), which increases strongly the viscosity of the starch suspension. With further heating, the starch forms a viscous mass or paste as a result of complete granule swelling and disruption (BeMiller and Huber, 2008).

Furthermore, the increase of temperature generates gluten coagulation, and subsequently the formation of large gluten aggregates (see 3.3.3. Gluten proteins functionality in the baking stage).

In addition to these changes, yeasts contain different enzymes responsible for the metabolism of microorganisms that modify dough characteristics and bread quality. Therefore, wheat flour and yeasts are sources of different endogenous enzymes in bread making processes and exert an important effect on dough rheology and on the technological quality of bread (Rosell and Benedito, 2003). These enzymes can be also added as processing aids to improve the quality of the baked products by reinforcing the role of gluten, providing fermentable sugars, and/or contributing to stabilize the hydrophobic-hydrophilic interactions (Rosell and Collar, 2008).

The characteristics of the gas bubbles released during fermentation and baking stage have a crucial impact on the final bread quality in terms of texture and final volume (Cauvain, 2003). During baking, as the heat penetrates the dough, the gases inside the dough expand and consequently the dough size increases. As the temperature rises, the rate of fermentation and production of gas cells increases, and this process continues until the temperature of yeast inactivation is reached (approximately 45 °C). When proteins are denatured, the gluten strands surrounding the individual gas cells are transformed into the semi-rigid structure that will yield the bread crumb. While the sugars and breakdown products of proteins released from the enzyme activity are then available to sweeten the bread crumb and participate in Maillard reaction or nonenzymatic browning reactions, which are responsible for the brown color of the crust (Mondal and Datta, 2008, Rosell, 2011).

The organoleptic characteristics of the bread are also affected by the peptides and amino acids contents of LMW proteins, released during fermentation and baking (Martinez-Anaya, 1996). During bread making amino acids are absorbed by yeast and metabolized as a nitrogen source for growth, resulting in an increase in the amount of gas produced, raising the alcohol tolerance of yeast and improving the organoleptic and nutritional quality of bread. The amino acids can also be hydrolysed by the action of proteolytic enzymes from flour as well as by yeast autolysis. Consequently, the total amino acid content (particularly for ornithine and threonine) increases by 64% during mixing and then decreases to reach 55% during baking, with the most reactive amino acids being glutamine, leucine, ornithine, arginine, lysine, and histidine (Prieto et al., 1990). Free amino acids in wheat flour and dough play an important

role in the generation of bread flavor precursors through the formation of Maillard compounds during baking (Martinez-Anaya, 1996).

Other constituants affect also the baking performances through several reactions and interactions. Although lipids are present only as minor constituent in wheat flour, they greatly impact the baking performances and small variations can affect wheat quality attributes (Koehler and Wieser, 2013). Previous studies (Hoseney et al. 1970) reported that the free polar fraction of non-starch lipids (principally glycolipids) are bound to the gliadin proteins by hydrophilic bonds and to the glutenin proteins by hydrophobic bonds and have a positive effect on baking performance, while the nonpolar fraction have the opposite effect (MacRitchie, 1984). The presence of polar lipids with the simultaneous binding to gliadins and glutenins improves dough properties, thus the gas holding capacity during proofing is increased, which contributes to increased loaf volume, to better crumb resilience, and, in some cases, to retardation of bread staling (Selmair and Koehler, 2008; Koehler and Wieser, 2013).

3.3. Gluten protein functionality in bread making

Notwithstanding the roles of non-gluten proteins and the other constituents of wheat flour in bread making process, gluten proteins remain the main quality determinants of bread making (Eliasson and Larsson, 1993; Veraverbeke and Delcour, 2002). The gluten proteins properties have the unique ability to allow wheat flour to be transformed into dough with appropriate properties for bread making. Therefore, gluten proteins undergo several changes during the different stages of bread making process.

3.3.1. Gluten proteins functionality in the mixing stage

For the he gluten network development, the native proteins of wheat flour require hydration and mechanical energy input, to modify the initial conformation of gliadin and glutenin chains (Wieser, 2007; Schiedt et al., 2013). When the water is added to the wheat flour, the gluten proteins unfold, favouring the heterogeneous distribution of the water around the long protein chains, especially around the polar amino acids and the charged ionisable groups (Schiedt et al., 2013). While mixing and by means of shear and tensile forces, gluten proteins join together and form a complex network in the dough (Singh and MacRitchie, 2001). The transformation of gluten proteins into a continuous cohesive visco-elastic gluten protein network occurs, when the native links of gluten forming proteins are broken and new bonds are formed, resulting in a protein matrix that is much stronger than the protein clusters in the wheat flour before hydration and mixing (Schiedt et al., 2013). Figure 7 shows a molecular

interpretation of the gluten network formation according to Shewry et al., (2002), Delcour et al., (2012), and Belton (1999). Gluten network is stabilized by intra- and intermolecular interactions of gliadins and glutenins (Wieser 2007; Schiedt et al., 2013). These interactions consist mainly on the disulphide bonds, formed by cross-links between cysteine residues through the disulphide-sulfhydryl exchange (Lindsay *et al.*, 2000; Tilley et al., 2001). Non-covalent interactions, especially, hydrogen bonds, hydrophobic interactions and electrostatic interaction stabilise also the gluten network (Belton 1999, 2005; Wellner et al., 2005; Barth 2007; Schiedt et al., 2013; Wang et al., 2015). These formed bonds provide extensibility, and elasticity characteristics.

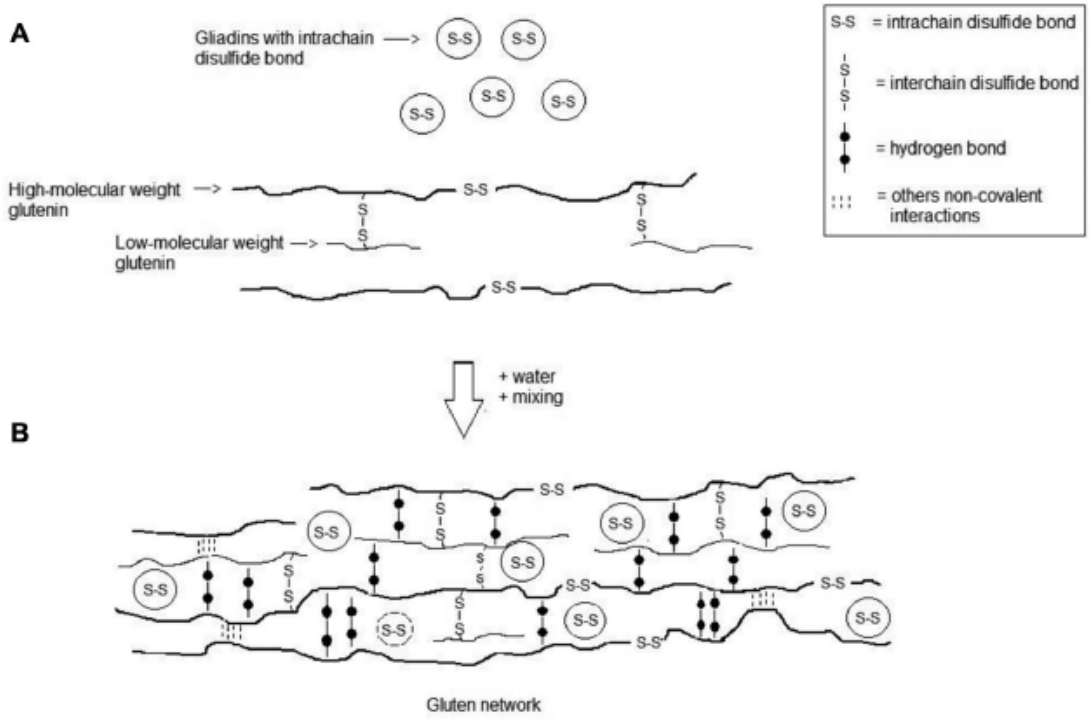


Figure 7: The molecular interpretation of the gluten network formation according to Abang-Zaidel et al., (2009), Shewry et al., (2002), Delcour et al., (2012), and Belton (1999).

As reported by Shewry et al., (2002); Don et al., (2003a, b); Wieser (2007); Wang et al., (2015), gluten network elasticity is mainly attributed to the disulphide bonds among the individual chains of glutenins. In this way, glutenin polymers are known to form a continuous network that provides strength (resistance to deformation) and elasticity to the dough (Belton, 1999), while the monomeric gliadins are believed to act as plasticisers of the glutenin polymeric system (Cornec et al., 1994; Khatkar et al., 1995).

3.3.2. Gluten proteins functionality in the fermentation stage

The gluten protein network plays a major role in retaining the carbon dioxide occluded in gas bubbles and prevents their coalescence that may occur during fermentation and at the beginning of the baking stage due to inappropriate viscoelastic properties of the gluten network in the dough. The gas retention capacity determines loaf volume and crumb structure of the resulting bread (Cauvain 2015; Hamer et al., 2009). Furthermore, gliadins can stabilise gas cells during bread making due to its surface-active properties, foam where the lipids in the dough are not able to cover the entire gas cell surface (Hamer et al., 2009; Wang et al., 2015).

3.3.3. Gluten proteins functionality in the baking stage

In this stage significant changes occur in the gluten proteins, due to heat. When the bread bakes, its gluten proteins coagulate (Wang et al., 2015). These gliadins and glutenins coagulation or denaturation is considered the most important change that occurs during baking. As observed by Schofield et al, (1983), gluten solubility and extractability decrease during heating, indicating increased cross-linking and polymerization of the gluten polymer, which was attributed to increased sulfhydryl–disulphide interchange reactions. Gluten surface hydrophobicity undergoes also changes, indicating unfolding of the gluten polymer exposing hydrophobic groups and decreasing solubility (Guerrieri et al., 1996). These reactions set the gluten, so that it is no longer elastic and determines the loaf volume, and bread characteristics.

As reported by Finny et al. (1948), wheat flour bread making performances are linearly related with flour protein content and with gluten protein content, because this protein fraction increases much more than the nongluten protein fraction with increasing grain protein content. However, this linear relationship between protein content and bread making performances depends on each variety, suggesting that gluten protein “quality” is also relevant. Thus, gluten proteins quantity and quality are both important characteristics for bread making.

Gluten protein quality is assumed to be determined by gliadins / glutenins ratio and the quality of the glutenin fraction (Goesaert et al., 2006). In addition to the balance required between dough viscosity (gliadins) and elasticity/strength (glutenins) for bread making quality, glutenin properties (composition, structure and molecular size distribution) have a crucial impact in explaining gluten protein quality differences in bread making (Goesaert et al., 2006). Knowing that more than 20 different HMW-GS and more than 40 different LMW-GS (Gupta and Shepherd, 1990) have been detected so far in different wheat varieties, this

explains an enormous variation in glutenin composition between different wheat varieties, which may result in differences in the noncovalent interactions that determine the elasticity of glutenin. Moreover, differences in the structure of glutenin largely affect glutenin functionality in bread making, which may also result from differences in glutenin composition (Goesaert et al., 2006). Finally, according to the molecular size distribution of glutenins, it was reported that only large glutenins polymers with certain molecular size distribution would contribute to the elasticity of the glutenin polymer network (MacRitchie, 1992; Singh and MacRitchie, 2001). This outcome was confirmed by other studies (Singh and MacRitchie, 2001; Veraverbeke and Delcour, 2002), highlighting positive correlations between dough strength/bread making performance and levels of the unextractable glutenin fractions known as the largest glutenin polymers.

4. Assessment of wheat baking quality

On the different levels of wheat bread production and processing, assessment and prediction of baking quality of wheat flour is of utmost interest. On the one hand, breeders in their selection process need to discern the wheat varieties that will fulfil the demands of the processing industry after approximately fourteen years of breeding (Lindhauer, 2014). On the other hand, cereal traders, millers and bakers need also to determine the optimal wheat material, required for the products ordered with a specific baking quality (Lindhauer, 2014). Baking quality is an important and complex character of soft wheat (*T aestivum*) (Pomeranz, 1988). Its definition differs largely between the countries, since the consumer's demands and the bread making technology vary according to cultural traditions (MacRitchie, 1984; Mesdag, 1985). However, several methods for determining the baking quality have been developed and standardized through the world, in particular by the International Association for *Cereal* Science and Technology (*ICC*) and the American Association of *Cereal* Chemists (AACC), the International Standardisation Organisation (ISO) and the European Committee for Standardization (CEN) (Freund and Kim, 2006; Lindhauer, 2014). These methods are classified into direct method, which consist on baking tests and indirect methods, including flour analytic methods as well as rheological methods.

4.1. Direct methods

Baking tests, which present the direct methods, are considered as the most accurate way to assess and provide straightforward and reliable information about wheat baking quality, under

standardized conditions. Generally, the volume of the loaf is taken as the single factor on which judgement is based (MacRitchie, 1984). However, other parameters of baked products are also evaluated: form, browning, crispness, crumb structure and elasticity, and finally taste. Several standardized baking tests methods are used to determine baking properties, like for example: the baking test of wheat flours according to ICC Standard Method 131. This baking test method enables comparability throughout the world and is characterised by high speed dough mixing and a short fermentation time (Freund and Kim, 2006). Whereas baking test method for pan bread and for rolls have proved very satisfactory in the assessment of wheat baking quality for the European countries (Freund and Kim, 2006).

However, baking tests require specific and expensive equipment of baking facilities, experienced staff, time, and a considerable quantity of wheat material, which is an inappropriate condition in early stages of breeding.

4.2.Indirect methods

Several indirect methods are used for the estimation of the wheat baking quality and can be classified into quantitative and qualitative methods. Some of these methods were applied in the current study, and are as follows:
Moisture content, ash content, falling number, crude protein content, sedimentation value, Farinograph, Gluto-Peak®-Test and Extensograph. In addition to these procedures, an extraction and quantification of gluten macro polymer was also carried out in order to assess the bakinq quality.
A detailed description of the used methods is provided in the next chapter: materials and methods.

4.3.Baking quality classes

The wheat is divided into subclasses based on characteristics influencing the quality of end product, and according to the system of each country (Lásztity and Salgó, 2003).

Table 3: German classification system of baking quality classes (Federal Plant Variety Office, 2015)

Baking quality classes	Description	Characteristics	Application
E-class	Elite wheat	Excellent baking properties	Bread and bakery products
A-class	Quality wheat	Good baking properties	Bread and bakery products
B-class	Baking quality	Medium baking properties	Pastries
C-class	Other type of wheat	Poor baking properties	Not usable for baking

The German wheat classification system grade wheat varieties according to their baking quality, particularly loaf volume, as well as crude protein content, sedimentation value, falling number, water absorption and milling yield, and finally the comparison with the quality traits of a defined reference variety (Bundessortenamt 2015; Laidig et al., 2016). According to the German Descriptive Variety List, four wheat classes are known: E- class wheat (elite wheat) with the highest quality, A-class wheat (quality wheat), B-class wheat (baking wheat), and C-class with the lowest quality, which is also not usable for baking (Table 3).

Chapter 2

Materials and methods

1. Experimental plan

This study aims the assessment of the baking quality of soft wheat flour samples (*Triticum aestivum* L.), through the estimation of the loaf volume by means of new methods. Therefore, the thesis was subdivided into three main work packages. According to the objectives of each part, specific procedures were implemented. Table 4 summarizes the procedures used and the plant material investigated commensurate with the purpose of each work package.

Table 4: The plant material investigated, and the procedures applied according to the objectives of the work packages of the thesis

Work package	Objective	Plant material	Procedure
Work package 1: Prediction of bread wheat baking quality using an optimized GlutoPeak®-Test method.	- Optimisation of the GPT method. - Determination of the relationship between the loaf volume and the gluten aggregation properties, on the basis of a multigenotype and multilocation analysis. - Prediction of loaf volume of wheat flour samples.	- 15 different bread wheat varieties from the harvest year 2013, cultivated at 8 different locations in Germany (n = 120). - 11 different bread wheat varieties from the harvest year 2014, cultivated at 8 different locations in Germany (n = 88). - 21 different bread wheat varieties from the harvest year 2015, cultivated at eight different locations in Germany (n = 208).	- Determination of the protein content, sedimentation value and the loaf volume by means of the baking test. - Run of the GPT with different solvent/flour ratio, and different solvents. - Analyse of the GPT results and determination of the coefficients of correlation between the GPT parameters, the quality parameters and the loaf volume. - Multiple regression analysis to predict the loaf volume and validation of the best fit equation with an independent data set.
Work package 2: Relationship between glutenin macro polymer and bread wheat baking quality on the basis of multilocation and multigenotype analysis.	- Determination of the GMP and GMP subunits concentrations of wheat flour samples, highlighting the effect of quality class, genotype and location of growth on these concentrations. - Investigation of the relationship between GMP, GMP subunits concentrations and loaf volume on the basis of a multigenotype and multilocation analysis.	- 15 different bread wheat varieties from the harvest year 2013, cultivated at 8 different locations in Germany (n = 120). - 11 different bread wheat varieties from the harvest year 2014, cultivated at 8 different locations in Germany (n = 88).	- Determination of the protein content, sedimentation value and the loaf volume by means of the baking test. - Extraction of GMP and GMP subunits under reducing conditions and their quantitation by means of HPLC. - Statistical analyse of the results obtained, highlighting the variation of the parameters measured according to quality classes, location and genotype. - Determination of the coefficients of correlation between GMP, GMP subunits concentrations, quality parameters and loaf volume.

Work package 3: Prediction of blending effect and baking quality of soft wheat blends by means of Extensograph, Gluten Peak Tester and glutenin macro polymer under variable fertilisation levels and environmental conditions.	- Analyse of the BEV of flour blends according to genotype, location of growth and fertilisation level. - Investigation of the relationship between the aggregation gluten properties, the rheological dough properties, the GMP and GMP subunits concentrations and BEV of flour blends. Prediction of BEV and loaf volume of flour blends.	- Six different soft wheat varieties from the harvest year 2015 cultivated at three different locations in Germany and with three different levels of nitrogen fertilisation (n = 54). - Blend flour samples from the harvest year 2015 cultivated at three different locations in Germany and with three different levels of nitrogen fertilisation (n = 135).	- Determination of the protein content, sedimentation value and the loaf volume by means of the baking test. - Run of the GPT with the optimised method in work package 1. - Extraction of the GMP and GMP subunits under reducing conditions and quantification by means of HPLC. - Evaluation of the rheological dough properties through Extensograph tests. - Statistical analysis to emphasize the variation of BEV according to genotype, location of growth and fertilisation level. - Determination of the coefficients of correlation between all the parameters measured and BEV and loaf volume. - Multiple regression analysis to predict the BEV and the loaf volume of flour blends and evaluation of the best fit equation with a cross validation.

The plant material investigated, the experimental methods used as well as the statistical methods applied are clarified in the following sections.

2. Plant material

All the samples investigated in the present thesis are soft winter wheat varieties (*Triticum aestivum*. L) and representatives of the commercial varieties that are actually registered in the German Descriptive Variety List (Beschreibende Sortenliste (BSL) vom Bundessortenamt).

For the first work package, 15 different soft wheat varieties from the harvest year 2013, 11 different soft wheat varieties from the harvest year 2014, and 21 different soft wheat varieties from the harvest year 2015 were used. Each soft wheat variety is cultivated at eight different locations in Germany, which represent the main German growing areas of winter wheat, with different soil and climate conditions. The plant material investigated in this first part thus consisted of 120, 88 and 208 different soft wheat flour samples from the harvest years 2013, 2014 and 2015, respectively.

For the second work package, the same varieties used in the first work package were considered, except soft wheat varieties from the harvest year 2015.

Finally, for the third work package six different soft winter wheat varieties from the harvest year 2015 cultivated at three different locations in Germany (from the federal states: Lower Saxony and Saxony-Anhalt) and with three different levels of nitrogen fertilisation (low, medium and high level of fertilisation) were investigated. These varieties received the same amount of nitrogen fertilizer at the beginning of the vegetation (50 Kg N/ ha), but different late-season nitrogen fertilisation levels, low (160 Kg N/ ha), medium (180 Kg N/ ha) and high (200 Kg N/ ha). These pure varieties were blended to each other, within location and level of fertilization after milling into flour to a ratio of 50:50, which resulted in 135 blend wheat flour samples in addition to the initial 54 pure wheat flour samples.

All wheat varieties samples used in the present study are classified according to the German Descriptive Variety List in three quality groups: Elite wheat (E-class), Quality wheat (A-class) and Bread wheat (B-class). Only these three quality groups were included in this study, while C-class, which is not useable for baking was not involved. Table 5 summarised the number of pure wheat varieties per location according to the quality classes used in the present study.

Table 5: Number of pure wheat varieties per location according to the quality classes.

Work package	Harvest year	Quality class	Number of varieties per location
Work package 1: Prediction of bread wheat baking quality using an optimized GlutoPeak®-Test method.	2013	E A B	4 8 3
	2014	E A B	2 1 8
	2015	E A B	2 12 7
Work package 2: Relationship between glutenin macro polymer and bread wheat baking quality on the basis of multilocation and multigenotype analysis.	2013	E A B	4 8 3
	2014	E A B	2 1 8
Work package 3: Prediction of blending effect and baking quality of soft wheat blends by means of Extensograph, Gluten Peak Tester and glutenin macro polymer under variable fertilisation levels and environmental conditions.	2015	E A B	1 3 2

3. Experimental methods

All wheat varieties were milled into flour using a Buehler laboratory mill MLU 202. Flour type 550 was produced according to DIN 10355 Standard with approximately 0.60 % ash content, as recommended by Arbeitsgemeinschaft Getreideforschung, (2016).

The ash content was determined according to ICC Standard Method 104/1. Samples are weighed (5 – 6 g) into crucibles and placed into a muffle furnace at 900 °C for 2 hours. The residue is cooled in a dessicator and then weighted to be expressed as a percentage of the initial samples weight.

When a sample is incinerated in an ash oven, the high temperature drives out the moisture and burns away all the organic materials (starch, protein, and lipids), leaving only the ash. The residue is composed of the non-combustible, inorganic minerals that are concentrated in the bran layer (WMC, 2004).

Ash content is an extremely important measurement to achieve specified ash levels of wheat flour types. Ash content is also an indication of the yield that can be expected during milling, since it is primarily concentrated in the bran.

3.1. Determination of moisture content

Determining moisture content is an essential first step in analysing wheat flour quality, since it is used for other tests. Moisture content of 14 % is commonly used as a conversion factor for other tests in which the results are affected by moisture content. Moreover, it represents also an indicator of the grain shelf life. Wheat or flour with high moisture content, over 14.5 % attracts mold, bacteria and insects, which cause deterioration during storage (WMC, 2004).
In the present study, the moisture content of the whole wheat grain was determined according to the DIN EN 15948:2012 with the Near Infrared Transmission spectroscopy (NITs) with Infratec™ 1241(Foss GMbH, Rellingen, Germany).

3.2. Determination of crude protein content

The crude protein content was determined using NITs with Infratec™ 1241(Foss GMbH, Rellingen, Germany), according to DIN EN 15948:2012. It is expressed in percentage, on a dry matter basis.
Crude protein content as an indirect parameter for the assessment of baking quality is globally still the main criterion in evaluating wheat baking quality, because gluten proteins are recognized as the most important components governing bread-making quality (Wrigley and Bietz, 1988). However, protein content cannot be considered as a single parameter to characterize bread-making quality of wheat flour, since it is largely determined by the quality of its protein components (Caballero et al., 2007).

3.3. Determination of sedimentation value

The swelling properties of wheat flours are tested by determining the sedimentation value, which is an approximate measure of baking quality. Since the gluten proteins are insoluble in water, the sediment volume indicates the amount of gluten and reflects the swelling properties of the gluten. That means the sedimentation value as indirect parameter to assess the wheat baking quality combines qualitative and quantitative measurement of the wheat gluten (Freund and Kim, 2006).

Zeleny Sedimentation value was determined according to ICC 116/1, after preparing the flour samples according to ICC 118. 3.2 g of wheat flour with a moisture content of 14 % are combined with 50 ml of bromophenol blue solution in a sedimentation cylinder, shaken by hand for 5 s and then by the mixing rank for 5 min. Subsequently, 25 ml of the sedimentation test reagent, consisting of lactic acid, isopropyl alcohol and distilled water, are added and returned to mixing rack for a further 5 min. The cylinder is left stand without moving for exactly 5 min, then the volume of the sediment obtained indicates the sedimentation value. The sedimentation value is expressed in ml. Sedimentation value will range from 8 ml for a very low protein content, weak gluten type, to about 78 ml for a very high protein content and strong gluten type.

3.4. Baking test: Rapid Mix Test

The baking test used in the current study is the Rapid Mix Test (Pelshenke et al., 2007); as a standard baking method to produce bread rolls to measure the loaf volume for flour type 550. The name of this baking test is due to the quick method of mixing and making of bread rolls. Moreover, this method is based on the automation of most activities, in order to limit the human influence on the results (Freund and Kim, 2006). Before the dough is prepared, the moisture content of samples of wheat flour type 550 is determined with the NIT Spectroscopy of the whole grain with Infratec™ 1241(Foss GMbH, Rellingen, Germany) according to the DIN EN 15948:2012, as previously clarified. Then the weight of flour is corrected to a moisture content of 14 %. The falling number is determined according to Hagberg-Perten DIN EN ISO 3093: 2009 and adjusted to a falling number of 250 ± 25 s by adding malted wheat flour. Whereas for flours with high enzyme activity, that means falling number below 200 s, no malt flour is added. Finally, using the Farinograph (Brabender GmbH and Co KG, Duisburg, Germany) according to ICC 115/1, the water absorption (ml/100 g) is determined at a consistency of 500 BU. Subsequently, the dough is prepared from wheat flour (1000 g), yeast (5 %), salt (1.5 %), sugar (1 %), peanut fat (1 %) and ascorbic acid (0.002 %). The temperature of the dough must be between 26 °C and 27 °C, while the temperature of the water added is the difference between the sum of the flour and room temperature, and the key number determined by means of the comprehensive instructions of the baking test. The ingredients are mixed for 1 min; interrupt after 20 s to scrape the sides of the mixing bowl. The dough has to be kneaded and shaped within the resting time in order to reduce the time between mixing and baking, thus no more than one hour separates the end of mixing and oven charging. The dough pieces are divided and rolled into balls by means of a divider, and then

after an intermediate fermentation an elongated shape is produces of the dough pieces by a moulder. The rolled dough pieces are left to rise for 25 min before oven charging. Before baking, the dough pieces are left for 2 min at room temperature to stiffen and finally baked at 250 °C for 20 min (Pelshenke et al., 2007; Freund and Kim, 2006).

- Determination of the falling number

The falling number consists of a measure of alpha-amylase activity of the wheat flour.
The determination of the falling number was accomplished according to Hagberg- Perten DIN EN ISO 3093:2009. 7 g of wheat flour are combined with 25 ml of distilled water in a glass falling number tube with a stirrer and shaken and then heated in a water bath for one minute at approximately 95 °C, in order to permit the starch gelatinisation and the formation of a gel. The time required to stir and allow a visometer stirrer to sink through the gel to the bottom of the measuring tube is the falling number value. Falling number results are recorded as an index of enzyme activity in a wheat flour sample and the results are expressed in time as second. The results obtained from this test are used in the baking test.
A high falling number (for example, above 300 seconds) indicates minimal alpha-amylase activity and sound quality wheat flour. This too much activity results in sticky dough during processing and poor texture in the finished product. In this case, enzymes can be added to the flour in various ways to compensate.
A low falling number (for example, below 250 seconds) indicates substantial enzyme activity and sprout-damaged wheat flour. In this case, enzymes cannot be removed from the flour, which makes the flour unusable (WMC, 2004; Freund and Kim, 2006).
This falling number was determined in the present study, due to its necessity to perform the baking test.

- Determination of the water absorption by means of Farinograph

In order to estimate the amount of water required to make dough in the baking test and the Extensograph in the present study, Farinograph test was performed using the Farinograph (Brabender GmbH and Co KG, Duisburg, Germany) according to ICC 115/1.
A flour sample of 300 g with a moisture content of 14 % is placed in the Farinograph mixing bowl. Water from a buret is added to the flour and mixed until the dough reaches a consistency of 500 BU. The amount of water added is a measure of the water absorption of

the flour, since a consistency of 500 BU corresponds to the average firmness of dough (Freund and Kim, 2006).

3.5. GlutoPeak®-Test

The assessment of the gluten aggregation properties was carried out using the GlutoPeak®-Test (Brabender GmbH and Co KG, Duisburg, Germany) (Figure 8).
In order to optimise the standard GPT method and enable a separation between the investigated varieties, different solvents were used, and different amounts of flour and solvent were added into the sample cup at 36 °C, and the mixing paddle was operated at a speed of 2750 rpm.
The solvents used are: Distilled water, 5.0 % sucrose solution (w/w), 5.0 % (w/w) sodium carbonate solution and 5.0 % (w/w) lactic acid solution and were prepared according to AACC Method 56-11.02. In addition to these solvents, 1.0 % sodium chloride solution (w/w) was also used and prepared according to Marti et al. (2015a, b). 9 g flour was dispersed in 9 g solvent in the case of distilled water, sucrose solution, sodium carbonate solution and lactic acid solution, while 10.2 g flour was added to 11.2 g sodium chloride solution.
During the GPT test, a high energy is introduced into the flour-solvent suspension, which incites the gluten to agglomerate and develop a resistance against the mixing paddle. This resistance is measured as torque and demonstrated as a curve highlighting the build-up and the disaggregation of the gluten network.

The parameters defining this build up are automatically measured by the GPT software, and are as follows:
Maximum Torque (MT) expressed in Brabender Equivalents (BE) corresponding to the peak caused by aggregated gluten, Peak Maximum Time (PMT) expressed in seconds corresponding to the time at peak torque, AM and PM expressed in BE corresponding to the torque 15 s before the PMT and to the torque 15 s after the MT, respectively, and the five areas expressed in arbitrary units (AU) and corresponding to the energy required for gluten aggregation; A0-A1 is corresponding to the area under the curve from the beginning of the test until the first maximum, A1-A2 is corresponding to the area under the curve from the first maximum to the first minimum after first maximum, A2-A3 is corresponding to the area under the curve from the first minimum after first maximum to AM, A3-A4 is corresponding to the area under the curve from AM to MT, A4-A5 is corresponding to the area under the curve from MT to PM.

All measurements were performed for 300 s in duplicates.

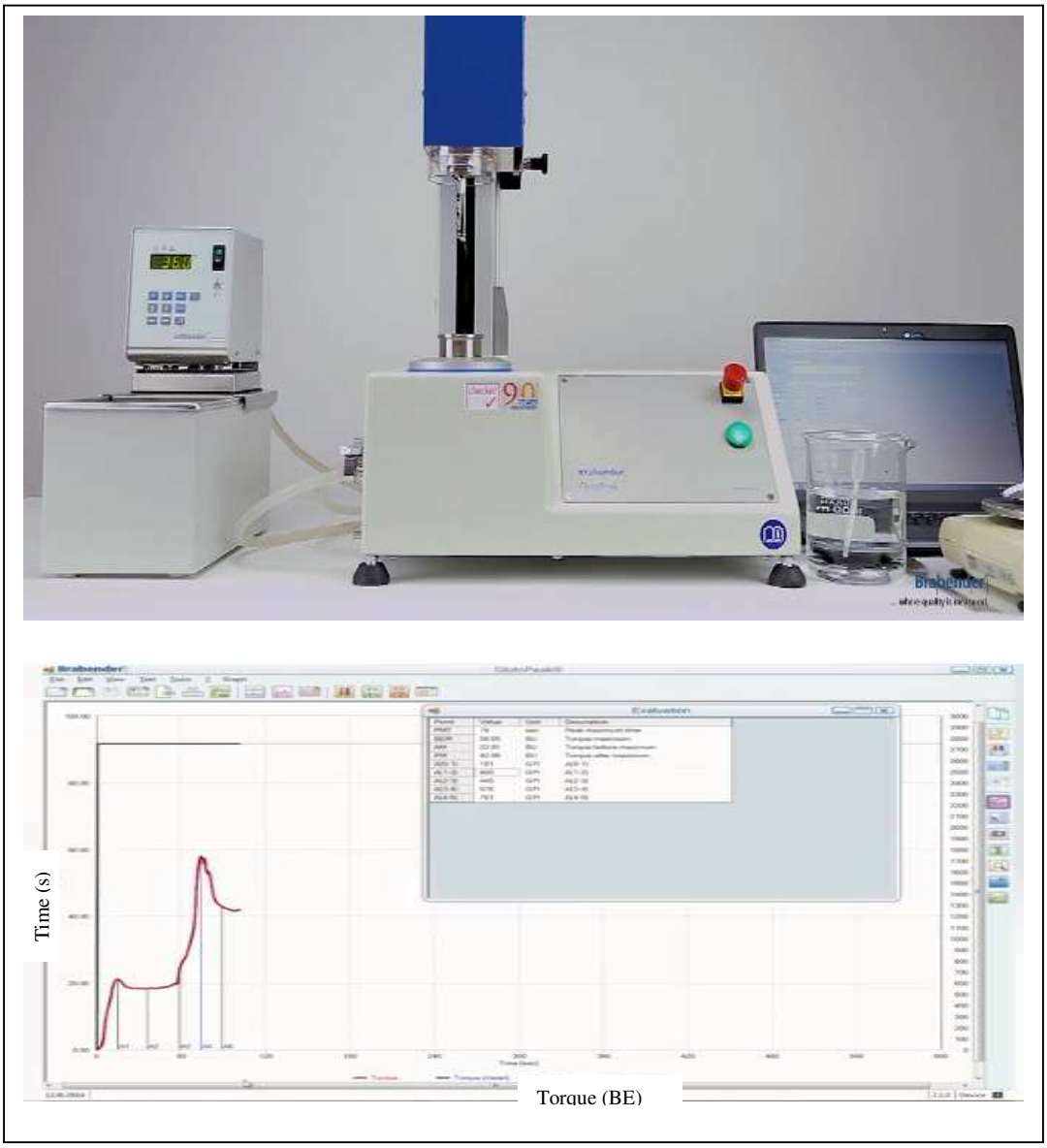


Figure 8: GlutoPeak®-Test and example of the curve obtained by the GPT software (Brabender GmbH and Co KG, Duisburg, Germany).

3.6. Extraction and quantitation of GMP and GMP subunits

GMP of flours were extracted and quantitated by means of HPLC following the procedure described by Thanhaeuser et al. (2014). The procedure of extraction is summarised in Figure 9 as a schematic representation.

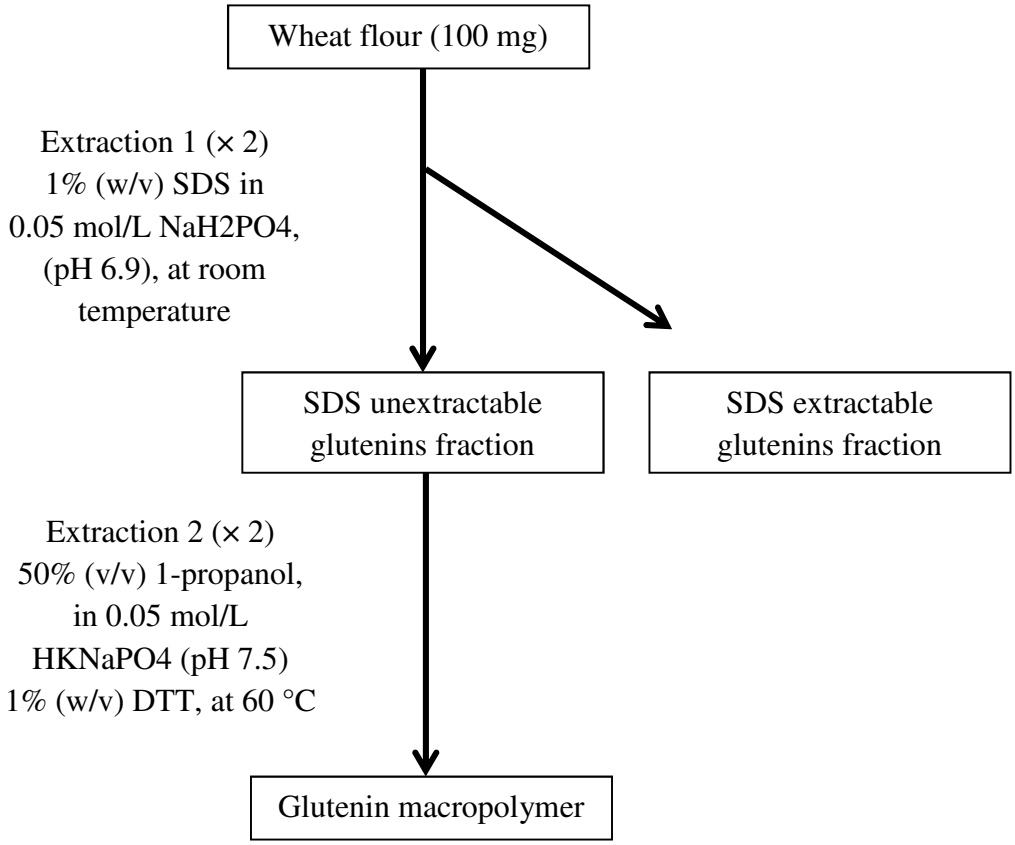


Figure 9: Schematic representation of the method applied for the extraction of the glutenin macropolymer (GMP) fraction according to Thanhaeuser et al. (2014).

Different chemicals were required for the extraction of GMP:
SDS Pellets ≥ 99,5% Rotiphorese-Grade, Hydrochloric acid fuming (HCl) ROTIPURAN® 37%, p.a., and 1,4-Dithiothreitol ≥ 99% (DTT), p.a., were from Carl Roth GmbH + Co KG (Karlsruhe, Germany), di-Sodium hydrogen phosphate dehydrates (NaH2PO4), Potassium dihydrogen phosphate (HKNaPO4) and 1-Propanol for analysis EMSURE® ACS,Reag. Ph Eur. ≤ 100% were purchased from Merck KGaA (Darmstadt, Germany).
100 mg of flour was weighted into a Pyrex tube (capacity: 12 ml), then first extracted twice at room temperature with extraction solvent A (1% (w/v) SDS and 0.05 mol/L NaH2PO4, (pH 6.9)), in order to separate the SDS extractable glutenin fraction. The pH value of the solvent A was adjusted with HCl (32%, w/w). This first extraction was initiated by vortexing for 2 min after addition of 1 ml of solvent A, and then followed by magnetic stirring for 30 min at room temperature. Afterwards a centrifugation of the suspension at 20 °C for 25 min at 4600 rpm/min was run (Heraeus Megafuge 1.0, Thermo Fisher Scientific, Dreieich, Germany). The supernatant containing the SDS extractable glutenin fraction was removed by means of a

pipette, and the precipitate was extracted twice for the second time using the extraction solvent B (50% (v/v) 1-propanol, 0.05 mol/L HKNaPO4 (pH 7.5) and 1% (w/v) DTT).
1 ml of the extraction solvent B was added to the precipitate, which was blanketed with nitrogen for 15 seconds. Afterwards it was vortexed for 2 min and then extracted with stirring for 30 min at 60°C in a water bath. After cooling of the Pyrex tube in cool running water, the suspension was centrifuged at 20 °C for 25 min at 4600 rpm/min. The supernatant containing the GMP was transferred to a volumetric flask (capacity: 2 ml) and diluted to 2 ml with solvent B. The extracts were filtered through a 0.45µm cellulose acetate membrane filter and transferred into the HPLC vials.
A Kontron HPLC instrument (Kontron Instrument, Roßdorf, Germany) fitted with a Yarra 3u SEC-3000 column (Phenomenex, Aschaffenburg, Germany) was used to quantify the GMP and GMP subunits under the following conditions: The column temperature was the room temperature, the injection volume was 20 µL, the eluent was solvent A at a flow rate of 0.3 ml/min, and detection was made by measuring the UV absorbance at 210 nm by means of a Kontron 332 UV-Vis-Detektor. The column used has a length of 300 mm and an internal diameter of 4.6 mm.
As a reference material for the calibration, gliadin reference from the Prolamin Working Group (PWG gliadin) (van Eckert et al., 2006) was used. For this purpose, dilution series was prepared with a range of concentrations 0 µ*g*/ml, 50 µ*g*/ml, 100 µ*g*/ml, 100 µ*g*/ml, 250 µ*g*/ml, 500 µ*g*/ml, 1000 µ*g*/ml, 1500 µ*g*/ml and 2000 µ*g*/ml using the solvent B as a dilution solution. These standard solutions were also transferred into the HPLC vials and analysed by means of the HPLC in the same manner as for the GMP quantification. A calibration curve was defined by means of the peaks areas obtained of the respective concentrations. The equation of this calibration curve enabled the determination of the GMP and the GMP subunits concentrations from their obtained peaks areas.
The integration of the obtained areas was performed using Chromgate software (Knauer, Berlin, Germany).
The extractions and measurements were carried out for each flour sample in duplicate.

3.7. Extensograph

The Extensograph has been demonstrated as a useful equipment to determine the resistance and extensibility of a dough by measuring the force required to stretch the dough with a hook until it breaks. This test is accomplished according to the Standard Method ICC 144/1. In addition to distilled water and 2 % Sodium chloride, 0.002 % Ascorbic acid was added to

300g wheat flour (14 % moisture content) in the determination of the necessary amount of water to achieve a dough consistency of 500 BU after 5 min mixing using the Farinograph (Brabender GmbH and Co KG, Duisburg, Germany) and according to ICC 115/1. After mixing and modelling, the dough piece secured with pins into the Extensograph cradle is rested for 45 min. then a hook is drawn through the dough, stretching it downwards until it breaks. As the test is run, a curve is recorded on graph paper. This same dough piece is shaped and stretched two more times, after 90 min and 135 min resting time.

Extensograph results include resistance to extension, extensibility, and area under the curve.

Resistance to Extension is a measure of dough strength. A higher resistance to extension requires more force to stretch the dough. It is known as R value and is indicated by the maximum height of the curve. It is expressed in Brabender units (BU).

Extensibility indicates the amount of elasticity in the dough and its ability to stretch without breaking. It is the E value and is indicated by the length of the curve. It is expressed in millimetres (mm).

Energy is indicated by the area under the curve which is a combination of resistance and extensibility. It is expressed in square centimetres (cm^2).

R/E Ratio indicates the balance between dough strength (resistance to extension) and the extent to which the dough can be stretched before breaking (extensibility).

Results from the Extensograph Test are useful in determining the gluten strength and bread making characteristics of flour (WMC, 2004).

All measurements in the present study were performed for each sample in duplicate.

4. Statistical methods

All statistical analysis was performed using SigmaPlot for windows version 11 and 14 (Systat Software Inc., San Jose, USA).

To highlight the relationship between the different parameters investigated in the current study the linear Pearson correlation was performed.

To test whether or not the means of several independent groups of observations are significantly different, the Analysis of Variance (ANOVA) was used as a parametric statistical approach in the case of normal distributed data, or the Kruskal-Wallis test as a nonparametric test in the case of non-normal distributed data (distribution free). The ANOVA was carried out using mean values of the measured parameters, while the Kruskal-Wallis test was performed by means of the median values. In the two cases, if $p \leq 0.001$, there is a significant difference between the groups compared. Subsequently, a multiple comparison

statistical procedure by means of Post Hoc Test was used to highlight where the differences occurred between groups at a significance level of $\alpha = 0.05$ (values with $p < 0.05$ were considered significantly different). In work package 2, Dunn's Test was used as Post Hoc Test because it is the only Post Hoc Test available after a Kruskal Wallis Test when the sample sizes are not equal. However, in work package 3, Holm-Sidak Test was carried out as Post Hoc Test.

Finally, to develop a predictive model for the dependent variables, best subset regression analysis was applied.

Chapter 3

Prediction of bread wheat baking quality using an optimized GlutoPeak®-Test method

I. Introduction

On different levels of soft wheat production and utilisation, it is fundamental and of utmost interest to predict the baking quality of the raw material. At early stages of soft wheat production chain, the breeders need to assess the baking quality of wheat varieties they work with, in order to determine if these varieties after release will fulfil the requirements of the bakers and millers after many years of breeding. On the other hand, millers and bakers are always facing the challenge of selecting the appropriate material to meet the customers demand. Therefore, since many decades cereal scientists have elaborated a system of methods suitable to assess the baking quality of soft wheat varieties (Freund and Kim, 2006; Lindhauer, 2014).

Among these methods, baking tests were and are still considered as the most accurate way to mirror and assess wheat baking quality. The volume of the loaf generated from the baking test is generally taken as the single factor on which the baking quality determination is based. However, baking tests necessitate specific equipment of baking facilities, experienced staff, time and a considerable quantity of wheat material, which is an inappropriate condition in early stages of breeding. Moreover, the variability that can occurs during the baking test execution, and the dough over- or under-kneading can generate differences in the baking quality assessment (Canja et al, 2014).

Consequently, the grain protein content, as an indirect parameter for baking quality, turned out to be the most relevant criterion in evaluating wheat baking quality, since it showed a good linear relationship with the loaf volume, and the gluten proteins are recognized as the most important components governing bread-making quality (Wrigley and Bietz, 1988). However, in recent years and at least in Germany some approved wheat varieties do not have a linear dependency between protein content and loaf volume. Those varieties have relatively low grain proteins contents; however, because of the high functionality of the gluten proteins, the baking performance of such wheat varieties is much better than expected from classical quality parameters (Lindhauer, 2014). These observations highlight the fundamental impact of gluten proteins quality on dough forming and baking quality, and that gluten quantity cannot be considered as a single parameter to characterize bread-making quality of wheat flour (Caballero et al., 2007). As a result, breeders, millers and bakers are claiming that those

classical quality parameters are no longer suitable to predict the baking quality of modern German wheat varieties (Seling, 2010; Kazman 2010).

Therefore, a reliable assessment of baking quality of soft wheat flour samples by means of new method is nowadays of major importance for breeders and millers, as well as for bakers. Consequently, current studies focus on investigating new methods to assess loaf volume and baking quality, on the basis of gluten performances.

Among these methods, and in addition to the chemical predictive tests, like e.g., gluten index, SDS sedimentation test, Zeleny sedimentation test and solvent retention capacity, several rheological methods like Farinograph, Alveograph and Extensograph tests have been also used to evaluate the gluten strength and predict dough properties prior to processing and bread quality (Dowell et al., 2008; Ktenioudaki et al., 2010). Some of them (such as Farinograph and Mixograph) determine, the amount of mixing that dough requires or the amount of water that should be added to the flour to obtain dough of the desired consistency. Others simulate the rounding and moulding in the baking process and measure the dough resistance to extension (e.g. Extensograph, Alveograph. Those methods remain reliable techniques in quality testing; however, these methods are time consuming, labour intensive and require large simple sizes of about 50 g to 300 g of flour (Kaur and Seetharaman, 2011), which is not in line with the actual needs along the value chain of wheat. Breeders look for reliable methods to test the functional quality of wheat lines at early stages with just a limited amount of sample. The milling industry needs fast and reliable methods for checking wheat quality right at the receiving station. Finally, the baking industry is looking for suitable methods that could predict end product quality for production and product development (Marti et al, 2015a).

The GlutoPeak®-Test has been recently proposed for the quick evaluation of wheat baking quality by measuring the aggregation behavior of gluten (Kaur and Seetharaman, 2011). GPT is a rapid shear-based method for measuring the gluten aggregation properties. During the test, the gluten of the flour forms a network and develops a resistance against the mixing paddle. The resistance is measured as torque and demonstrated as a torque-time curve. Small sample size (3-10 g), results within some minutes (1-10 min), easy handling are the key elements which deserve all the attention given to study the ability of the GPT to assess the wheat gluten quality. This test has been already proposed as a valid screening tool for durum wheat quality (Marti et al., 2013, 2014). GPT indices were also recommended for predicting conventional wheat parameters related to dough mixing stability, extensibility, and tenacity (Marti et al., 2015a). Nevertheless, all of these studies have in common that they have tested

the GPT method either with a restricted number of samples or within a single-year analyses, which could only provide an indication of the applicability of the tested method, as no estimates of the interactions over different years and interaction of genotype-by-location are available. However, genotype by environment interaction is an important issue as both contribute equally to an optimal evaluation of a new method, due to the fact, that the various components of flour protein differ in their response to environmental and genotype influence and will change according to location, cultivating conditions and season (Kaya and Akcura, 2014).

Accordingly, the purpose of this study was to optimize the GPT method for a precise and universally usable application for predicting wheat quality on the basis of a multiyear and multilocation analysis. Therefore, the solvent/flour ratio as a relevant parameter has been varied and different solvents as alternative to pure water have been evaluated. Finally, correlations were established between the loaf volume and the gluten aggregation behavior of flours from a set of different winter wheat varieties harvested at 8 different locations in Germany over two years. Multiple regression analysis was used to investigate the relationship between loaf volume and various screening tests and the equation with the best fit was applied to an independent data set from the harvest year 2015.

II. Article 1

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Prediction of bread wheat baking quality using an optimized GlutoPeak®-Test method

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Abstract

The GlutoPeak®-Test (GPT) as a rapid small-scale technique was optimized to evaluate the gluten aggregation properties and to predict the loaf volume, on the basis of a multiyear and multilocation analysis of wheat samples, using different solvents. 5 % lactic acid and 1 % sodium chloride displayed significant GPT responses. Relationships between protein content, sedimentation value, GPT parameters and loaf volume were investigated. With 1 % sodium chloride, the torque 15 s before maximum torque (AM) presented the highest correlation with loaf volume of samples from 2013 and 2014 ($r = 0.77$, $r = 0.63$, $p < 0.001$, respectively). A multiple regression analysis indicated that the best prediction of loaf volume was a linear function of protein content and AM, explaining the variation in loaf volume by 63 % and

providing an uncertainty of +/- 39 ml. The accuracy of the validation of the linear function leads to 64 % correct and to 36 % incorrect predictions of the loaf volume. This emphasizes that the application of the linear function of protein content and AM cannot replace the actual measurement of loaf volume, but it could be a useful rapid screening test in breeding for improved baking quality in bread wheat.

Keywords: GlutoPeak®-test (GPT); Protein content; Sedimentation value; Loaf volume.

1. Introduction

Wheat (*Triticum aestivum* L.) is one of our most important food crops, and it is a major source of energy, protein, and several micronutrients. Based on statistics from the FAO, it can be estimated that per year around 450 million tons of wheat are used worldwide for food, making it to our quantitatively most important cereal food together with rice (Svihus, 2014). Its importance is mainly due to the fact that its kernel can be ground into flour, semolina, etc., which form the basic ingredients of bread and other bakery products, as well as pasta (Šramková et al., 2009), therefore the greatest proportion of wheat flour produced is used for bread making.

Baking tests are still considered as the most accurate way to assess wheat baking quality and the volume of the loaf is generally taken as the single factor on which judgement is based. Disadvantages of baking tests are that specific equipment of baking facilities, experienced staff and time are required, so that they are not suited for quick routine analyses. Therefore, the grain protein content, as an indirect parameter for baking quality, is globally still the main criterion in evaluating wheat baking quality, because gluten proteins are recognized as the most important components governing bread-making quality (Wrigley and Bietz, 1988). However, gluten quantity cannot be considered as a single parameter to characterize bread-making quality of wheat flour, since it is largely determined by the quality of its protein components (Caballero et al., 2007). It is a well-established understanding that the main components of the wheat protein, the glutenins and gliadins, have the highest impact on dough forming and baking quality (Khatkhar et al., 1995). But nevertheless, many studies have already demonstrated that large differences exist in the proportion of the variation in loaf volume that can be explained by the variation in protein content and quality (Koppel and Ingver, 2010). This was also demonstrated by some approved varieties in Germany which do not have a linear dependency between protein quantity and loaf volume. Those varieties have relatively low grain proteins contents; however, because of the high functionality of the gluten

proteins, the baking performance of such wheat varieties is much better than expected from classical quality parameters (Lindhauer, 2014).

Consequently, various quick methods have been tested to determine the gluten performance and baking quality. Among the chemical predictive tests are gluten index, sodium dodecyl sulphate sedimentation test, Zeleny sedimentation test and solvent retention capacity (SRC) (Kweon et al., 2011). Rheological methods like Farinograph, Alveograph and Extensograph tests have been also used to evaluate the gluten strength and remain reliable techniques in quality testing; however, these methods are time-consuming, labor intensive and require large sample sizes of about 50 g to 300 g of flour (Kaur and Seetharaman, 2011).

Recently the GlutoPeak®-Test (GPT) has been proposed for the quick evaluation of wheat baking quality by measuring the aggregation behavior of gluten (Kaur and Seetharaman, 2011). GPT is a rapid shear-based method for measuring the gluten aggregation properties. During the test, the gluten of the flour forms a network and develops a resistance against the mixing paddle. The resistance is measured as torque and demonstrated as a torque-time curve. Small sample size (3-10 g), results within some minutes (1-10 min), easy handling are the key elements which deserve all the attention given to study the ability of the GPT to assess the wheat gluten quality. This test has been already proposed as a valid screening tool for durum wheat quality (Marti et al., 2013, 2014). GPT indices were also recommended for predicting conventional wheat parameters related to dough mixing stability, extensibility, and tenacity (Marti et al., 2015a). Nevertheless, all of these studies have in common that they have tested the GPT method either with a restricted number of samples or within a single-year analyses, which could only provide an indication of the applicability of the tested method, as no estimates of the interactions over different years and interaction of genotype-by-location are available. However, genotype by environment interaction is an important issue as both contribute equally to an optimal evaluation of a new method, due to the fact, that the various components of flour protein differ in their response to environmental and genotype influence and will change according to location, cultivating conditions and season (Kaya and Akcura, 2014).

Accordingly, the purpose of this study was to optimize the GPT method for a precise and universally usable application for predicting wheat quality on the basis of a multiyear and multilocation analysis. Hence, the solvent/flour ratio as a relevant parameter has been varied. In addition, different solvents as alternative to pure water have been evaluated. As alternative solvents, lactic acid, sucrose in water and sodium carbonate in water were chosen as they are applied within the SRC profile (AACC 10-56). Those solvents were selected because of the

ability of the SRC to predict the functionality of different flour constituents through the solvents used (Duyvejonck et al., 2011). Moreover, we tested sodium chloride to highlight different functional aspects within the wheat flour. When sodium chloride is present during hydration and mixing, it shields the charges on the gluten protein, reducing electrostatic repulsion between proteins and allowing them to be more closely associated (Miller and Hoseney, 2008). Finally, correlations were established between the loaf volume and the gluten aggregation behavior of flours from a set of different winter wheat varieties harvested at 8 different locations in Germany over two years. Multiple regression analysis was used to investigate the relationship between loaf volume and various screening tests and the equation with the best fit was applied to an independent data set from the harvest year 2015.

2. Material and Methods

2.1. Plant material

This study included 15 different bread wheat varieties from the harvest year 2013,11 different bread wheat varieties from the harvest year 2014 and 21 different bread wheat varieties from the harvest year 2015, cultivated at eight different locations in Germany. The eight trial locations represent effectively the main growing areas of winter wheat in Germany. The wheat varieties are classified according to the German Descriptive Variety List in three groups: Elite wheat (E): n = 4 (2013), n = 2 (2014), n = 2 (2015), Quality wheat (A): n = 8 (2013), n = 1 (2014), n = 12 (2015) and Bread wheat (B): n = 3 (2013), n = 8 (2014), n = 7 (2015). Selected varieties are representatives of the commercial varieties that are currently registered in the German Descriptive Variety List.

2.2. Standard methods

Protein content was determined using Near Infrared Transmission spectroscopy (NITs) with Infratec™ 1241(Foss GMbH, Rellingen, Germany), according to DIN EN 15948:2012 (protein content % expressed on a dry matter basis). Zeleny Sedimentation value was determined according to ICC 116/1, after preparing the flour samples according to ICC 118.

2.3. Rapid Mix Test

Samples were milled into flour, using a Buehler laboratory mill MLU 202, to produce flour type 550 with approximatively 0.60 % ash content, determined according to ICC Standard Method 104/1. The Rapid Mix Test (Pelshenke et al., 2007) is used as a standard baking method to produce bread rolls in order to measure the loaf volume. Before the dough is

prepared, the moisture content was determined with the NIT Spectroscopy of the whole grain with Infratec™ 1241(Foss GMbH, Rellingen, Germany) according to the DIN EN 15948:2012, the falling number according to Hagberg-Perten DIN EN ISO 3093:2009, and finally the water absorption (ml/100g) determined using the Farinograph (Brabender GmbH and Co KG, Duisburg, Germany) according to ICC 115/1.

2.4. GlutoPeak®-Test

The assessment of the gluten aggregation properties was carried out using the GlutoPeak®-Test (Brabender GmbH and Co KG, Duisburg, Germany). Different amounts of flour and solvent were added into the sample cup at 36 °C, and the paddle was operated at a speed of 2750 rpm.

Distilled water, 5.0 % sucrose solution (w/w), 5.0 % (w/w) sodium carbonate solution and 5.0 % (w/w) lactic acid solution, were prepared according to AACC Method 56-11.02. In addition to the SRC solvents, 1.0 % sodium chloride solution (w/w) was also prepared.

9 g flour was dispersed in 9 g solvent in the case of distilled water, sucrose solution, sodium carbonate solution and lactic acid solution, while 10.2 g flour was added to 11.2 g sodium chloride solution. All measurements were performed for 300 s in duplicates.

The parameters measured automatically by the GPT software are:

Maximum Torque (MT) expressed in Brabender Equivalents (BE) corresponding to the peak caused by aggregated gluten, Peak Maximum Time (PMT) expressed in seconds corresponding to the time at peak torque, AM and PM expressed in BE corresponding to the torque 15 seconds before the PMT and to the torque 15 seconds after the MT, respectively, and the five areas expressed in arbitrary units (AU) and corresponding to the energy required for gluten aggregation; A0-A1 is corresponding to the area under the curve from the beginning of the test until the first maximum, A1-A2 is corresponding to the area under the curve from the first maximum to the first minimum after first maximum, A2-A3 is corresponding to the area under the curve from the first minimum after first maximum to AM, A3-A4 is corresponding to the area under the curve from AM to MT, A4-A5 is corresponding to the area under the curve from MT to PM.

In addition, for the method using 5 % lactic acid area A7 was calculated using Microsoft Excel 2010 and is corresponding to the area under the curve from the beginning of the test to the minimum of the slope (point Ay), when the gluten network was completely disaggregated (Fig. 1a). Furthermore, for the method using 1 % sodium chloride the following areas expressed in AU were also calculated using Microsoft Excel 2010: the area Ax-A4 is

corresponding to the area under the curve from the gluten aggregation starting point until MT, area A6 is corresponding to the area under the curve, located at the top of area A3-A5, i.e. the area defined by AM and PM and at the top, MT (Fig. 1b).

2.5. Statistical analysis

Statistical analysis (Pearson correlation coefficient and multiple regression analysis) were performed using SigmaPlot for windows version 11 (Systat Software Inc., San Jose, USA).

3. Results and Discussion

The wheat samples tested showed a large variation in the physico-chemical parameters measured:

The crude protein content ranges from 11.1-16.3 % DM in 2013 (10.4-15.6 % DM (2014)), the sedimentation value from 19-75 ml (21-75 ml (2014)) and the loaf volume from 480-783 ml/100g (460-800 ml/100g (2014)). For wheat flour samples from 2013 loaf volume showed a good correlation with protein content and sedimentation value (Table 1). A high correlation was observed between loaf volume of wheat flour samples from the harvest year 2014 and protein content ($r = 0.81$, $p < 0.001$). The latter correlation confirmed previous studies (Hruskova et al., 2001).

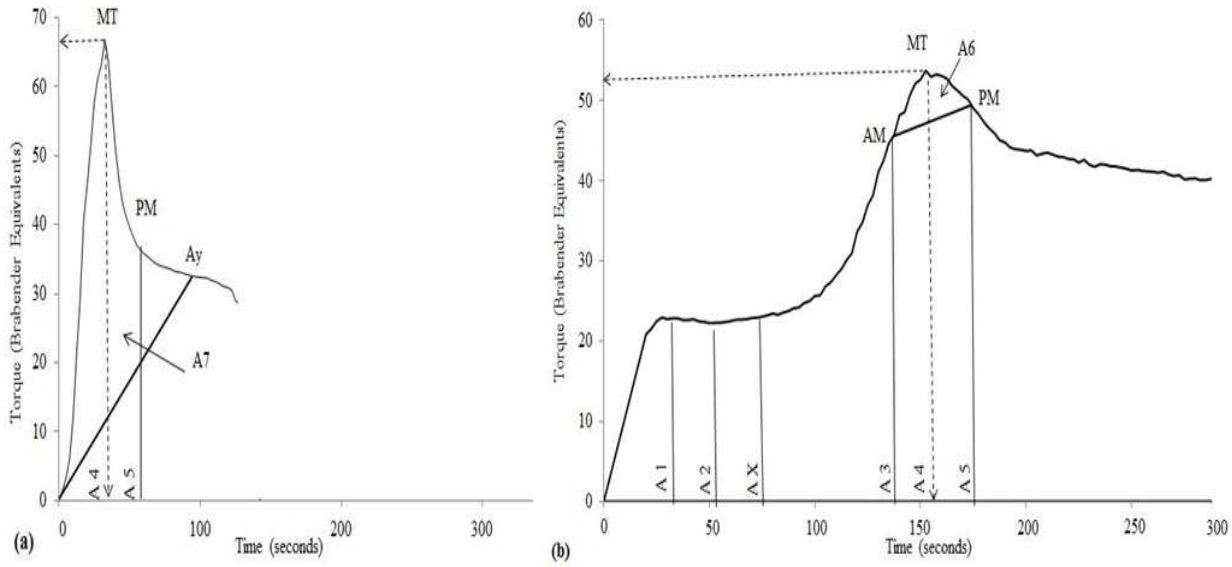


Figure 1: Example of the GPT monitoring curve of a wheat flour sample.

(a) Flour tested using 5% lactic acid. The GPT indices measured are: Peak maximum time (PMT), maximum Torque (MT), torque 15 seconds before the MT (AM), torque 15 seconds after the MT (PM), area from the beginning of the test and the first maximum (A0-A1), area from the first maximum and the first minimum after first maximum (A1-A2), area from the first minimum after first maximum and AM (A2-A3), area from AM and MT (A3-A4), area from MT and PM (A4-A5), area

from the gluten aggregation starting point and the MT (Ax-A4), A7area from the beginning of the test to the minimum of the slope.

(b) Flour tested using 1% sodium chloride. The GPT indices measured are: Peak maximum time (PMT), maximum Torque (MT), torque 15 seconds before the MT (AM), torque 15 seconds after the MT (PM), area from the beginning of the test and the first maximum (A0-A1), area from the first maximum and the first minimum after first maximum (A1-A2), area from the first minimum after first maximum and AM (A2-A3), area from AM and MT (A3-A4), area from MT and PM (A4-A5), area from the gluten aggregation starting point and MT (Ax-A4), area from AM and PM (A6).

3.1.Effect of distilled water, 5 % sucrose and 5 % sodium carbonate on gluten aggregation

Distilled water was used as a control in the first experiment to run the GPT, in order to evaluate the ability of this solvent to highlight the quality of wheat samples. Distilled water has an evident effect on flour samples since the gluten network formation was clearly demonstrated by the torque-time curve (data not shown). The results obtained showed different shapes of curves pointed out by PMT, MT and the five areas of the curve, however some flour samples had two peaks instead of one (data not shown) which suggest that distilled water as solvent does not classify the samples significantly.

5 % sodium carbonate was also used to test the samples with the GPT. The results obtained displayed no peak formation (data not shown). The effect of the pH on wheat proteins can explain these results, as explained by Hoseney and Brown, (1983). It can be attributed to a number of factors including modification of the structure of dough proteins through electrostatic attraction or repulsion resulting from changes in the degree of ionization of ionizable groups on flour proteins. A pH value of 11.6, in the presence of sodium carbonate provided strong repulsive forces between highly negative charges along gluten protein chains, which might be responsible for preventing the protein molecules from associating and forming networks (Gennadios et al., 1993).

Table 1: Coefficients of correlation (r) and level of significance (p) between protein content, sedimentation value, GPT parameters and loaf volume using 5 % lactic acid and 1% sodium carbonate as solvents for wheat flour samples from harvest year 2013 (n=120) and 2014 (n =88)

Parameters		Crude Protein content (% DM)	Sedime-ntation value (ml)	Solvent	PMT (s)	MT (BE)	AM (BE)	PM (BE)	A0-A1 (AU)	A1-A2 (AU)	A2-A3 (AU)	A3-A4 (AU)	A4-A5 (AU)	Ax-A4 (AU)	A6 (AU)	A7 (AU)
Flour samples 2013 Loaf volume (ml/100g) n = 120	r	0.65	0.79	**5% lactic acid**	0.52	0.60	-	0.67	-	-	-	-	0.70	-	-	0.67
	p	<0.001	<0.001		<0.001	<0.001	-	<0.001	-	-	-	-	<0.001	-	-	<0.001
Flour samples 2014 Loaf volume (ml/100g) n = 88	r	0.81	0.64		-0.14	0.54	-	0.50	-	-	-	-	0.56	-	-	0.51
	p	<0.001	<0.001		<0.001	<0.001	-	<0.001	-	-	-	-	<0.001	-	-	<0.001
Flour samples 2013 Loaf volume (ml/100g) n = 120	r	0.65	0.79	**1% sodium carbonate**	0.17	0.56	0.77	0.61	0.03	0.25	0.34	0.70	0.58	0.70	0.51	-
	p	<0.001	<0.001		n.s[(a)]	<0.001	<0.001	<0.001	n.s	0.007	<0.001	<0.001	<0.001	<0.001	<0.001	-
Flour samples 2014 Loaf volume (ml/100g) n = 88	r	0.81	0.64		-0.31	0.53	0.63	0.59	0.17	-0.17	-0.03	0.63	0.57	0.56	0.57	-
	p	<0.001	<0.001		<0.001	<0.001	<0.001	<0.001	n.s	n.s	n.s	<0.001	<0.001	<0.001	<0.001	-

[(a)] $p > 0.05$, not significant (n.s)

The addition of 5 % sucrose as a solvent to perform the GPT induces the formation of the gluten network which is demonstrated by the torques obtained (data not shown). Torques obtained using sucrose were higher than those obtained using distilled water, indicating a decrease of the water absorption of flour, thus less amount of water is required to develop the dough, as noted by Babajide et al. (2014) using honey. Moreover, like in the presence of distilled water, the GPT using 5 % sucrose does not enable a classification and a differentiation between the different flour samples based on quality classes, protein content and loaf volume.

3.2.Effect of lactic acid on the on gluten aggregation

Lactic acid contributes to obtain comparable shapes of curves for different samples tested. The gluten aggregated in the presence of lactic acid in less than 15 s meaning that AM and consequently the other areas of the curve were not measured. In this case, samples were differentiated only by PMT, MT, PM, A4-A5 and A7 (Fig.1a). The flour samples showed a rapid build-up of the gluten network, which led to a sharply defined peak followed by a rapid breakdown. These are characteristics commonly exhibited by weak flours with poor technological quality (Goldstein, 2010). This weakness was due to the presence of lactic acid. At pH below 4.0 there was a sizable positive charge and an increase in intramolecular electrostatic repulsion enhancing gluten proteins unfolding and increasing the exposure of their hydrophobic groups, but the presence of strong intermolecular electrostatic repulsive forces prevents the formation of new bonds. Moreover, at this low pH the proteolytic activity is further enhanced and might lead to the degradation of the gluten proteins responsible for the dough's firmness and elasticity resulting in a softer and less elastic gluten network (Clarke et al., 2004). But nevertheless, a good classification and a differentiation between the different flour samples based on quality classes, protein content and loaf volume was possible.

3.3.Correlation of gluten aggregation parameters and quality parameters with loaf volume using 5 % lactic acid as a solvent for the GlutoPeak®-Test

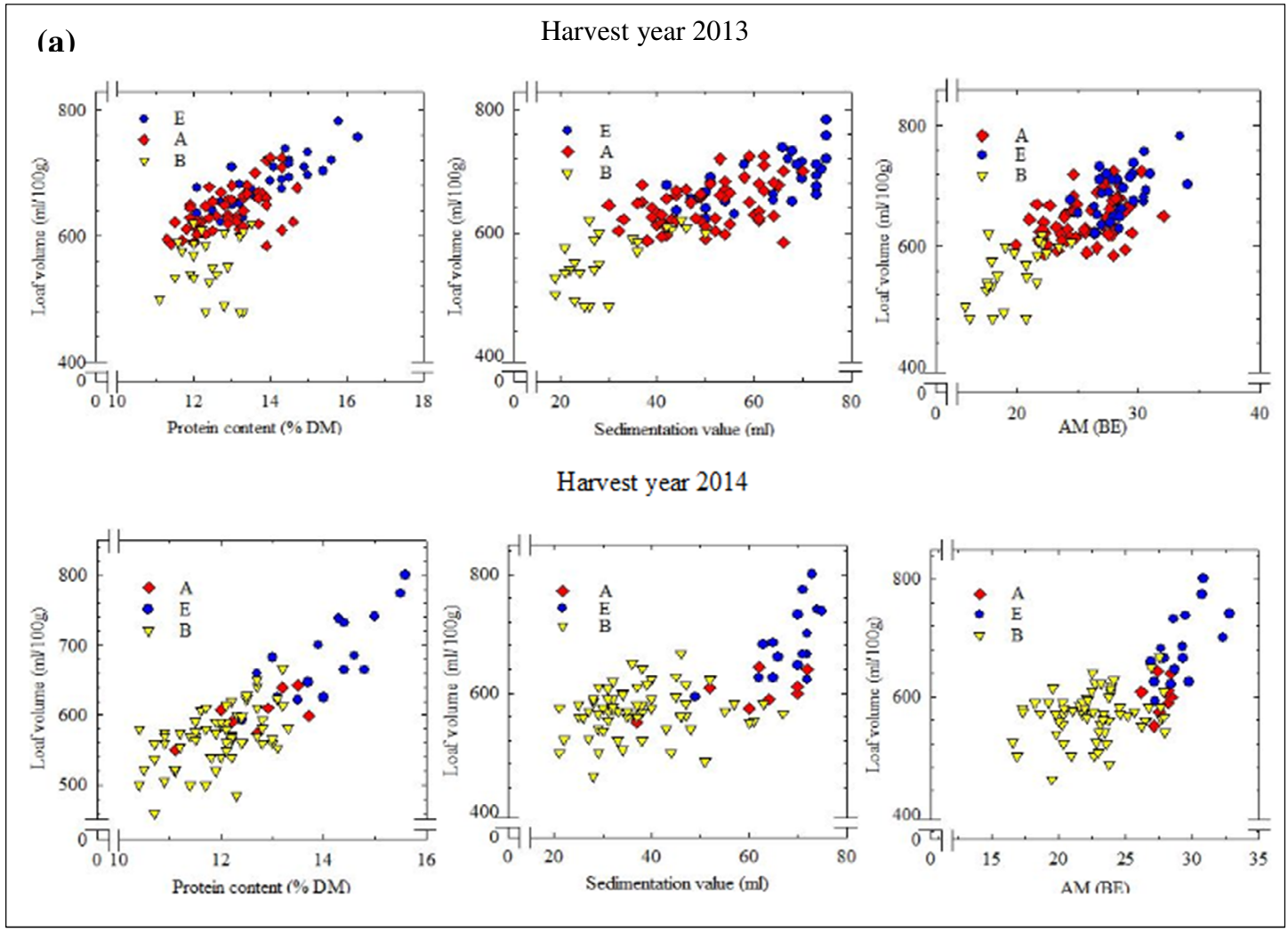
A linear Pearson correlation (r) was conducted to highlight the relationship between loaf volume, and the GPT parameters for samples of the harvest year 2013 and 2014. The outcomes are summarized in Table 1.

The gluten aggregation parameters, outlined by the GPT parameters, were positively correlated to the loaf volume. The area A4-A5 showed the highest correlation ($r = 0.70$, $p < 0.001$) in 2013. This high correlation led to the assumption that A4-A5 is a good parameter

representing the gliadin content of wheat samples, which means that a decrease in gluten strength caused by high gliadin content (high viscosity) require a high energy to disaggregate the gluten network formed. This outcome is confirmed by the study of Marti et al. (2015b), who showed a high correlation between the gliadin content and the loss of torque 30 s after gluten aggregation (corresponding to the decrease in torque 30 s after peak), approving that high gliadin content is negatively correlated with the gluten strength. However, the coefficient of correlation between loaf volume and GPT area A4-A5 does not reach the correlation values obtained between sedimentation value, protein content and loaf volume showed before in Table 1. Thus, the GPT using 5 % lactic acid cannot be considered as an optimal tool to replace both indirect methods: protein content and sedimentation value in the prediction of loaf volume.

Figure 2a shows the behavior of samples belonging to different wheat quality classes (E; A; B) in function to protein content, sedimentation value and GPT area A4-A5 against loaf volume of wheat flour samples from harvest year 2013 (n = 120) and 2014 (n =88). For each indirect method, samples of wheat E-class quality were superimposed to the samples belonging to wheat A-class, especially in the harvest year 2013. Whereas samples of wheat quality B-class were mostly separated from the other samples, positioned at lower values of protein content, sedimentation value, area A4-A5 and the loaf volume and staying together to form a cluster. Those findings demonstrated that the attribution of a given sample to a quality class is not straightforward, because wheat flour quality is a complex property that varies in a continuous manner and actually relies on several physical, chemical and rheological characteristics (Foca et al., 2007; Marti et al., 2015).

The correlation between protein content, sedimentation value, area A4-A5 and loaf volume for each location over two harvest years is summarized in Table 2.



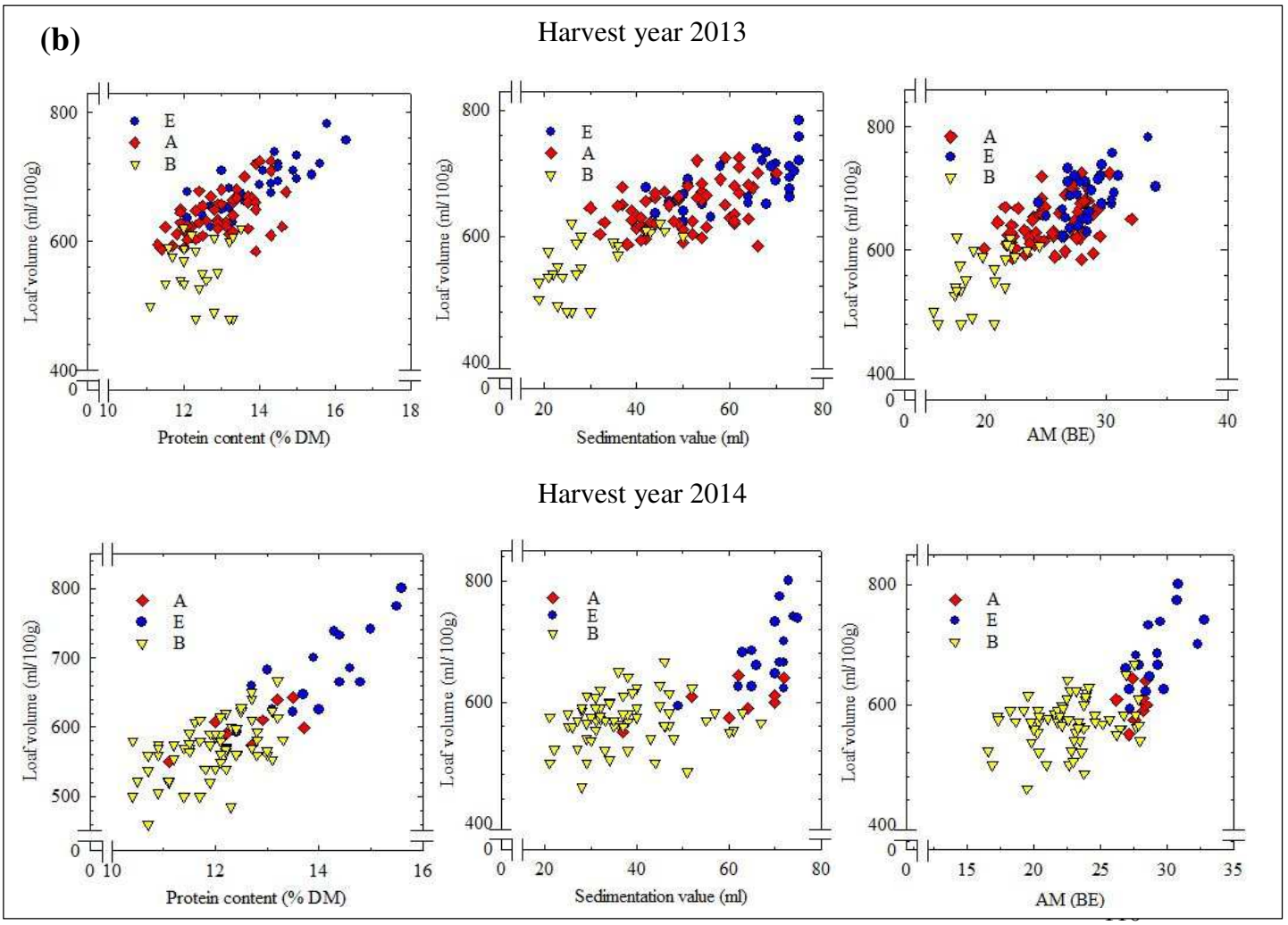


Figure 2: Relationship between protein content, sedimentation value, GPT area A4-A5 using 5% lactic acid (a), GPT area AM using 1% sodium chloride (b) and loaf volume for wheat flour samples belonging to different quality classes (E, A, B) and harvest years (2013 (n=120) and 2014 (n =88)).

Table 2: Coefficients of correlation (r) and level of significance (p) between protein content, sedimentation value, GPT area A4-A5 using 5 % lactic acid, GPT area AM using 1 % sodium chloride and loaf volume for wheat flour samples harvested at 8 different locations in Germany in 2013 (n=120) and 2014 (n =88)

Solvent	Harvest year	Location		Prot[a] / LV[b]	Sedi[c] / LV	A4-A5/ LV	AM / LV
5% lactic acid	2013	1	r	0.54	0.81	0.64	-
			p	0.04	<0.001	0.01	-
		2	r	0.75	0.86	0.58	-
			p	0.002	<0.001	0.02	-
		3	r	0.75	0.88	0.84	-
			p	<0.001	<0.001	<0.001	-
		4	r	0.78	0.90	0.92	-
			p	<0.001	<0.001	<0.001	-
		5	r	0.41	0.68	0.75	-
			p	0.13	0.01	<0.001	-
		6	r	0.63	0.78	0.73	-
			p	<0.001	<0.001	<0.001	-
		7	r	0.71	0.84	0.76	-
			p	<0.001	<0.001	<0.001	-
		8	r	0.76	0.91	0.77	-
			p	<0.001	<0.001	<0.001	-
	2014	1	r	0.83	0.55	0.60	-
			p	<0.001	n.s[d]	0.05	-
		2	r	0.74	0.42	0.35	-
			p	0.01	n.s	n.s	-
		3	r	0.70	0.62	0.31	-
			p	0.02	0.04	n.s	-
		4	r	0.72	0.72	0.42	-
			p	0.01	0.01	n.s	-
		5	r	0.83	0.51	0.53	-
			p	<0.001	n.s	n.s	-
		6	r	0.85	0.74	0.57	-
			p	<0.001	0.01	n.s	-
		7	r	0.86	0.83	0.74	-
			p	<0.001	<0.001	0.01	-
		8	r	0.91	0.73	0.73	-
			p	<0.001	0.01	0.01	-
		1	r	0.54	0.81	-	0.78
			p	0.03	<0.001	-	<0.001
		2	r	0.75	0.86	-	0.85
			p	0.002	<0.001	-	<0.001

1% sodium chloride	2013	3	r	0.75	0.88	-	0.88
			p	0.003	<0.001	-	<0.001
		4	r	0.78	0.90	-	0.87
			p	<0.001	<0.001	-	<0.001
		5	r	0.41	0.68	-	0.78
			p	n.s[(d)]	0.006	-	<0.001
		6	r	0.63	0.78	-	0.83
			p	0.01	<0.001	-	<0.001
		7	r	0.71	0.84	-	0.80
			p	0.003	<0.001	-	<0.001
		8	r	0.76	0.91	-	0.85
			p	<0.001	<0.001	-	<0.001
	2014	1	r	0.83	0.55	-	0.42
			p	0.002	0.07	-	n.s
		2	r	0.74	0.42	-	0.72
			p	0.009	n.s	-	0.01
		3	r	0.70	0.62	-	0.64
			p	0.01	0.04	-	0.03
		4	r	0.72	0.72	-	0.52
			p	0.01	0.01	-	n.s
		5	r	0.83	0.51	-	0.60
			p	0.002	n.s	-	0.05
		6	r	0.85	0.74	-	0.75
			p	<0.001	0.009	-	0.007
		7	r	0.86	0.83	-	0.74
			p	<0.001	0.002	-	0.009
		8	r	0.91	0.73	-	0.77
			p	<0.001	0.01	-	0.01

(a) Crude Protein content (% DM)
(b) Loaf volume (ml/100g flour)
(c) Sedimentation volume (ml)
(d) $p > 0.05$, not significant (n.s)

The ranges of correlation coefficients of flour samples from the harvest year 2013 obtained for the eight locations between the protein content, sedimentation value, GPT area A4-A5 and the loaf volume were: 0.41-0.78, 0.68-0.90, and 0.58-0.92, respectively. Location 4 showed the highest correlation between protein content, A4-A5 and the loaf volume, whereas location 8 had the best correlation between sedimentation volume and loaf volume. The correlation in 2014 between loaf volume and protein content ranged from 0.70 to 0.91, sedimentation value from 0.42 to 0.83 and A4-A5 from 0.31 to 0.74. The highest correlation between sedimentation value, A4-A5 and the loaf volume was reached in location 7, while location 8 displayed the highest correlation between loaf volume and protein content. This confirms that different correlation coefficients between the same parameters can be expected at different locations. In some locations (e.g. location 3 and 4, 2013; location 7 and 8, 2014), a good correlation between sedimentation value and loaf volume remained as an essential requirement to obtain a good correlation between A4-A5 and loaf volume too, however in other locations (e.g. locations 1 and 2, 2013; location 4 and 6, 2014), such a high correlation was not obtained, even if the interrelationship between sedimentation value and loaf volume was strong.

In summary, the highest correlation between the loaf volume and the GPT parameters using 5 % lactic acid was always achieved with the area A4-A5, but varying environmental condition means instability in the strength of the relationship between A4-A5 and the loaf volume, which was distinctly underlined in this study. Moreover, since the relationship between protein content, sedimentation value and loaf volume remains stronger as that obtained between A4-A5 and loaf volume, the GPT using 5 % lactic acid is not sufficient to characterize and predict baking quality in bread wheat varieties.

3.4. Effect of sodium chloride on gluten aggregation

This experiment exhibited the effect of 1 % sodium chloride on the GPT results. The solvent contributes to obtain a slow buildup in gluten network formation, compared to the result obtained using 5 % lactic acid, differentiated by PMT, MT, AM, PM, A0-A1, A1-A2, A2-A3, A3-A4, A4-A5, Ax-A4, and A6 (Fig.1b). The reason for delays in protein hydration could be explained by the assumption that salt draws away water molecules from gluten structures to interact with sodium and chloride ions, inducing a reduction in the hydration rate of the gluten, which results in the longer development time of dough during mixing, as it was shown from the previous work by McCann and Day, (2013).

3.5. Correlation of gluten aggregation parameters and quality parameters with loaf volume using 1 % sodium chloride as solvent for the GlutoPeak®-Test

The correlation coefficients and the level of significance for the relationship between the GlutoPeak parameters (PMT, MT, AM, PM, A0-A1, A1-A2, A2-A3, A3-A4, A4-A5, Ax-A4, A6) and the loaf volume are shown in Table 1.

The highest correlation ($r = 0.77$, $p < 0.001$, 2013 and $r = 0.63$, $p < 0.001$, 2014), was found between loaf volume and the GlutoPeak parameter AM. AM may reflect the glutenin content of wheat samples, glutenin is able to generate a cohesive structure of the gluten network, taking into consideration that an increase of glutenin content increases gluten strength, i.e. elasticity (Melnyk et al., 2012). The high molecular weight subunits of glutenin, which are relevant to elasticity, possess a number of cysteine residues available to form intermolecular cross links. This is because the degree of cross-linking will determine the bulk elastic properties. With a low degree of cross-linking the extensibility will be high, but with increased disulfide cross-linking the material would become more rubber-like (Shevry et al., 1991). Consequently, the higher the degree of cross linking is, the higher the AM value and the gluten network elasticity we get. This elasticity is increased in the presence of sodium chloride, in a way that dough shows higher resistance to extension as reported by McCann and Day (2013). The importance of glutenin content in increasing gluten strength was also highlighted by the study of Marti et al. (2015b), who obtained a significant correlation ($r = 0.68$, $p = 0.002$) between glutenin content and the GPT area Ax-A4.

This solvent was also a question of interest in the study of Marti et al. (2015a), who investigated 120 commercial wheat flours with the GPT using 0.33 mol/L sodium chloride, maintaining a ratio of 9 g of flour/10 ml of solvent and a paddle rotation speed of 3000 rpm. Among the GPT parameters, the area Ax-A4 (energy required for gluten aggregation) and the maximum torque exhibited the better correlation to the conventional parameters related to dough strength and extensibility (e.g. Alveograph W value, Resistance to extension, water absorption). The difference between our outcome and the results of this study may be the effect of the different conditions of the experiment (ratio of flour/solvent, mixing speed) and especially the concentration of sodium chloride. In the presence of a high concentration of this solvent (0.33 mol/L) electrostatic forces become negligible due to kosmotropic anions and cations of the salt which have an effect on water structure, and consequently, on gluten aggregation (Zhang and Cremer, 2006). Kosmotropes reinforce hydrophobic interactions between gluten proteins due to less water availability and result in less unfolding and

hydrogen bonding between unfolded chains. Subsequently more mixing time is necessary to enable the formation of inter-protein interactions through hydrogen bonding, hydrophobic interactions and inter-disulfide bonding that contribute to gluten development (Kinsella and Hale, 1984). Thus, in our study on account of the low sodium chloride (0.17 mol/L), the formation of the protein interactions took place in torque before maximum AM, which explain the good correlation between this parameter and the loaf volume. In the study of Marti et al. (2015a), the formation of these interactions is delayed and happened in the area Ax-A4 due to the high concentration of sodium chloride, resulting in a strong relationship between the area Ax-A4 and the conventional parameters related to dough strength and extensibility. This is also a valid point to explain the correlation founded between the same area Ax-A4 and the gluten macropolymer in the second study of Marti et al. (2015b), within 0.5 mol/L calcium chloride was chosen as a solvent to perform the GPT, maintaining a flour/solvent ratio constant and equal to 1.26 and a paddle rotation speed of 1900 rpm. The high concentration of calcium chloride has the same effect as the high concentration of sodium chloride on the protein interactions which delayed its formation.

Consequently, sodium chloride in low concentrations as a solvent to perform the GPT is needed to obtain suitable GPT parameters values enabling the correlation with the loaf volume. This outcome is different from the conclusion of Marti et al. (2015a), who suggested that water is also sufficient to gain satisfactory estimates of conventional parameters like dough strength.

Figure 2b displays the behavior of samples belonging to different wheat quality classes (E; A; B) in function to protein content, sedimentation value and GPT area AM against loaf volume of wheat flour samples from harvest year 2013 (n = 120) and 2014 (n =88).

Using 1 % sodium chloride as solvent for samples harvested in 2013 or 2014, wheat quality classes E and A were also superimposed, while class B was forming a cluster. Those results are consistent with the results using 5 % lactic acid and confirm the challenge to attribute a given wheat sample to a specific wheat quality class. As it has been noted, the distinction of wheat quality classes depended on several parameters that, when taken separately, did not allow a clean separation between the various classes and whose interpretation took implicitly into account complex interactions among all the parameters themselves (Foca et al., 2007).

The correlation between the loaf volume and protein content, sedimentation value and AM for each location over two harvest years is summarized in Table 2.

The correlations between protein content or sedimentation volume and loaf volume are in most cases higher than the correlation values obtained by means of GPT. Apart from that the coefficients of correlation between GPT area AM and loaf volume are often very high and stable over different locations (exception location 1 and 4, 2014), indicating that GPT with sodium chloride as a solvent measures protein quality rather than differences in protein content. They ranged in 2013 from 0.78 (location 1 and 5) to 0.88 (location 3), whereas r of flour samples from 2014 ranged from 0.42 (location 1) to 0.77 (location 8). These results are considered reliable because from 16 different locations over the harvest years 2013 and 2014, we obtained low coefficients of correlation between GPT area AM and loaf volume in just two locations (location 1 and 4, 2014). This outcome could not be highlighted without an extensive set of samples, which broadens the range of possible data, and gives a better understanding of the environmental and genotype influence. For example, in the study of Marti et al. (2015b), gluten macropolymer, which is considered as a predictor of baking performances (Thanhäuser et al., 2014), was correlated with the GPT area Ax-A4 of flour samples from the harvest year 2013 (n = 19, cultivated at different locations in the United States), and the coefficient of correlation obtained ($r = 0.78$, $p < 0.001$) was comparable with the results achieved in our study, when we correlated GPT parameter AM and loaf volume of flour samples from the harvest year 2013 (n = 120, cultivated at 8 locations in Germany). However, if a large set of samples from different harvest years were taken in the study of Marti et al. (2015b), maybe other coefficients of correlation would be obtained, as we remark when we investigate flour samples from the harvest year 2014 ($r = 0.63$, $p < 0.001$), which underlined the necessity to integrate years (seasonal variation) in method evaluation in order to offer a realistic idea of the predictive power on future unknown samples and the applicability in the baking industry.

In summary, genotype by environment interaction plays an important role in the ability of the GPT method using 1 % sodium chloride to predict loaf volume, with the major environmental influenced factor being protein content. Therefore, GPT results should not be interpreted as predictors of loaf volume unless the corresponding protein content is taken into consideration.

3.6. Prediction of loaf volume via GPT using 1 % sodium chloride and corresponding protein content using linear regressions

Predicting suitability of flour for various end users from one indirect method like protein content is not straight forward and hence a combination of two rapid tests is recommended to

assess baking quality. A multiple regression analysis with loaf volume as the dependent variable and all indirect tests (protein content, sedimentation value and GPT area AM) as independent variables was carried out and indicated that the best prediction of loaf volume was a linear function of protein content and the GPT area AM (Table 3).

Table 3: Prediction of loaf volume using best subsets regressions of indirect parameters

Model	n	Factors	RSQ [(a)]	S.E. [(b)]
1	208	Crude Protein content	0,56	42
2	208	Sedimentation value	0,51	45
3	208	Prot [(c)] + Sedi [(d)]	0,60	41
4	208	Sedi + AM	0,55	43
5	208	Prot + AM	0,63	39

[(a)] Coefficient of determination (R^2)
[(b)] Standard Error of Estimate
[(c)] Crude Protein content (% DM)
[(d)] Sedimentation volume (ml)

Summing up, GPT in combination with protein content (model 5) can explain the variation in loaf volume by 63 % and provides estimates of loaf volume within an uncertainty of +/- 39 ml (an uncertainty equal to +/- 6.5 % at a loaf volume of 600 ml /100g) which is better than the uncertainties of model 1, 2, 3 and 4 and comparable to the uncertainty of the reference method (+/- 36 ml).

As both, the number of locations and the number of testing years contribute equally to the accuracy of a method validation, we applied the equation with the best fit to an independent set of data (21 different bread wheat varieties from the harvest year 2015 cultivated at eight different locations in Germany). This resulted in 64 % correct (i.e. deviations are within the uncertainty of the reference method (+/- 36 ml is equal to +/- 6 % at a loaf volume of 600 ml /100g)) and in 36 % incorrect predictions (Fig. 3).
Those results indicate that the model created, didn't fit the data very well and it cannot be expected to replace the actual measurement of loaf volume, but nevertheless it could be a useful and rapid screening test in breeding for improved baking quality in bread wheat.

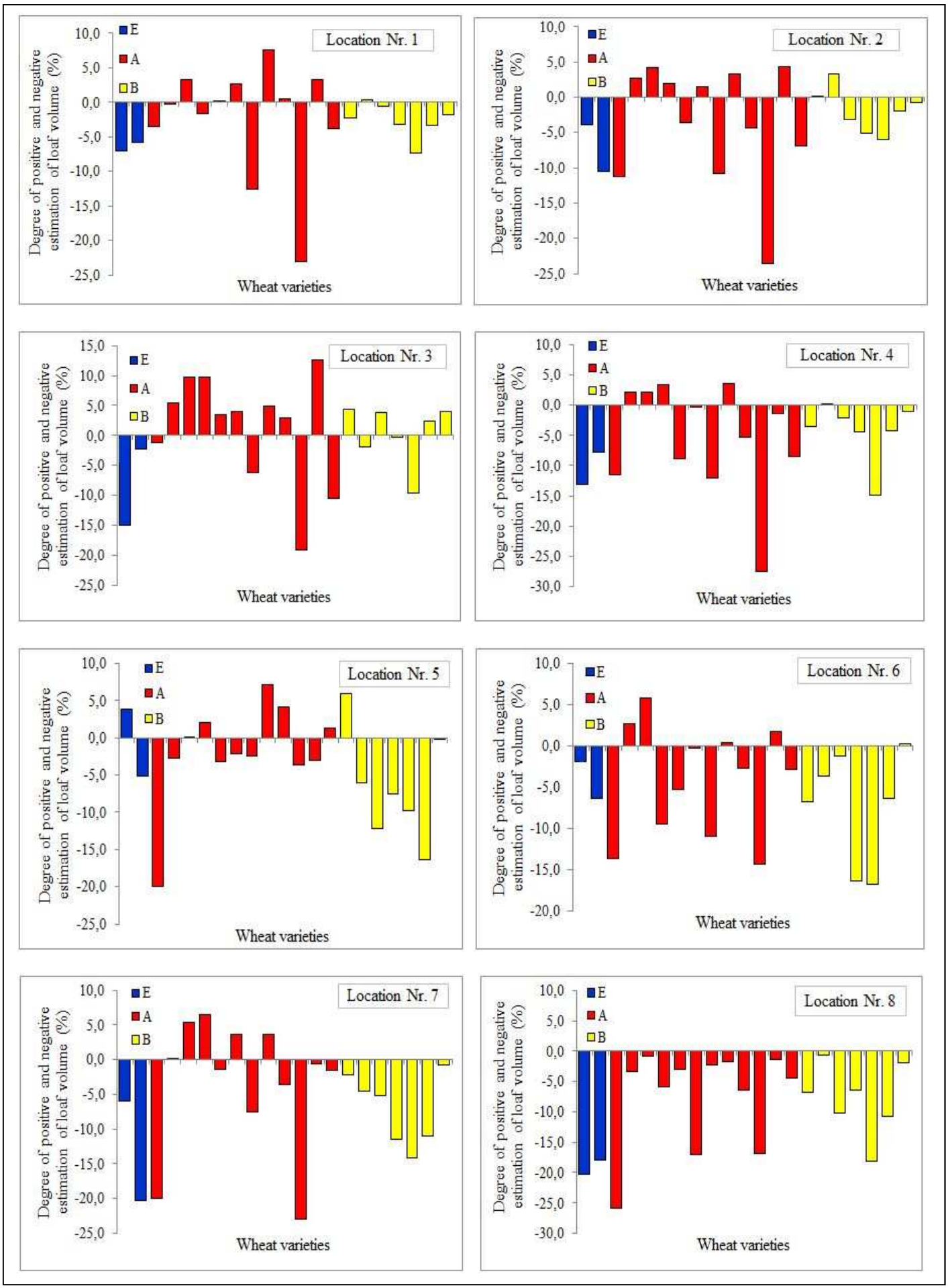


Figure 3: Degree of over- and underestimation of loaf volume for wheat flour samples belonging to different quality classes (E, A, B) from harvest year 2015 (n= 168).

4. Conclusions

This study demonstrates the GPT as a useful and reliable method to characterize wheat flours in accordance with their gluten aggregation, within some minutes and small sample size. Among the solvents used to run the GPT, the 1 % sodium chloride solution was the most convenient one to obtain a significant and environmental stable response regarding the loaf volume. Analysis exhibits that the influence of years on the stability of the method is of more importance than locations. A linear function of protein content and the GPT parameter AM was found to be useful to explain the variation in loaf volume over different location and year combinations. The evaluation of this linear function with an independent data set leads to 64 % correct and to 36 % incorrect predictions. This indicates that the strategy of combining two rapid tests is effective to a certain degree (e.g. for variety screening) and that the application of the model can be a useful approach in breeding for improved baking quality varieties in bread wheat, but it cannot be expected to replace the actual measurement of loaf volume via standard baking test.

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III. Discussion and conclusions

The objective of the current article was to investigate the ability of the GPT to assess the baking quality of soft wheat flour samples in different environmental conditions, for the purpose of predicting loaf volume. Therefore, an optimisation of the GPT standard method on the basis of a multiyear and multilocation analysis was carried out using different solvents and varying the solvent/flour ratio, whereas the temperature has been raised to 36 °C, and the mixing paddle was operated at a speed of 2750 rpm. The solvents used are: Distilled water, 5.0 % sucrose solution (w/w), 5.0 % (w/w) sodium carbonate solution, 5.0 % (w/w) lactic acid solution (SRC method solvents) and 1.0 % sodium chloride solution (w/w).

Among these solvents, only 5 % lactic acid and 1 % sodium chloride displayed significant GPT responses and enable a good classification and differentiation between the different flour samples investigated based on quality classes, protein content and loaf volume. With both solvents, the attribution of a given sample to a specific quality class (E, A and B quality classes) in function to protein content, sedimentation value and GPT parameters is not straightforward, confirming that wheat flour quality is a complex property that varies in a continuous manner and actually relies on several physical, chemical and rheological parameters, , when taken separately, did not allow a clean separation between the various classes and whose interpretation took implicitly into account complex interactions among all the parameters themselves (Foca et al., 2007; Marti et al., 2015a,b).

Using 5% lactic acid, the GPT parameter A4-A5 area presented the highest correlation values with loaf volume, which led to the assumption that this area represents the gliadin content of wheat samples. However, this correlation does not reach the correlation values obtained between sedimentation value, protein content and loaf volume and didn't remain stable over the different locations of growth over the two harvest years 2013 and 2014. This emphasises that varying environmental condition means instability in the strength of the relationship between A4-A5 and the loaf volume. Thus, the GPT using 5 % lactic acid cannot be considered as an optimal tool to replace the existing indirect methods in order characterize and predict baking quality in bread wheat varieties.

Using 1 % sodium chloride, the GPT parameter AM showed the highest correlation with loaf volume ($r = 0.77$, $p < 0.001$, in the harvest year 2013 and $r = 0.63$, $p < 0.001$, in the harvest year 2014). This observation leads to the assumption that this parameter may reflect the glutenin content of wheat samples, since glutenin is able to generate a cohesive structure of the gluten network, taking into consideration that an increase of glutenin content increases

gluten strength, i.e. elasticity (Melnyk et al., 2012). Consequently, it was reported in the current study that sodium chloride in low concentrations as a solvent to perform the GPT is needed to obtain suitable GPT parameters values enabling the correlation with the loaf volume.

The correlation between the GPT parameter AM and the loaf volume are often very high and stable over different locations, indicating that GPT with sodium chloride as a solvent measures protein quality rather than differences in protein content. That means genotype by environment interaction plays an important role in the ability of the GPT method using 1 % sodium chloride to predict loaf volume, with the major environmental influenced factor being protein content. Therefore, GPT results should not be interpreted as predictors of loaf volume unless the corresponding protein content is taken into consideration.

According to the results obtained, a combination of two rapid tests is recommended to assess baking quality and predict the loaf volume of soft wheat flour samples. Among the models determined by means of a multiple regression analysis with loaf volume as the dependent variable and all indirect tests (protein content, sedimentation value and GPT area AM) as independent variables, the best prediction of loaf volume was a linear function of protein content and the GPT parameter AM. This model can explain the variation in loaf volume by 63 % and provides estimates of loaf volume within an uncertainty of ±39 ml which is better than the uncertainties of the other models created and comparable to the uncertainty of the reference method (±36 ml). The validation of this model was carried out by means of an independent set of data. This resulted in 64 % correct and in 36 % incorrect predictions. Those results indicate that the model created, didn't fit the data very well and it cannot be expected to replace the actual measurement of loaf volume, but nevertheless it could be a useful and rapid screening test in breeding for improved baking quality in bread wheat.

Chapter 4

Relationship between glutenin macro polymer and bread wheat baking quality on the basis of multilocation and multigenotype analysis

I. Introduction

Gluten, as the main fraction of wheat flour proteins consists of monomeric gliadins and polymeric glutenins. The gliadins fraction has been reported to contribute to the viscous properties of wheat flour dough, and the glutenin fraction has long been considered to have a crucial role in dough strength and elasticity. A major part of the glutenin fraction is GMP, which may be defined as the large portion of the glutenin that is insoluble in an aqueous SDS solution. The GMP consists mainly of very large structures of HMW and LMW subunits linked by interchain disulphide bonds and have molecular masses roughly ranging from 5 to 20 million (Don et al., 2003a). Thus, they belong to the largest protein complexes in nature (Wrigley, 1996).

Detailed information on the composition and polymeric structure of the GMP are rare, and the positions of the polymerising interchain disulphide bonds are still unknown. Therefore, previous studies were aiming the optimisation of the GMP extraction method in order to get pure fractions and subsequently sufficient amount of GMP, which is necessary to characterise its composition (Moonen et al., 1982; Don et al., 2003a; Mueller et al, 2016).

According to those studies, GMP can be obtained as a gel by extraction of flour with a diluted SDS solution (e.g., 1.5 %). After centrifugation and decanting of the supernatant, the gel layer formed on the surface of the starch pellet is scraped off and washed with ethanol and water (Graveland et al., 1979; Moonen et al., 1982). Previous studies (Don et al., 2003a; Mueller et al, 2016) reported that this procedure results in the removal of most, but not all the soluble proteins, thus the GMP gel obtained contains a significant fraction of starch, and other monomeric proteins. According to this situation, in our current study the GMP was isolated by dissolving the SDS unextractable glutenin subfractions under reducing conditions (i.e, interchain disulfide bonds are broken) and subsequently, quantitated using gel permeation HPLC in order to obtain pure fractions (Cinco-Moroyoqui & MacRitchie, 2008; Cornec et al., 1994). The GMP analysis according this procedure could be also a useful tool for breeders in early stages of breeding, due to the very small amounts of material required (100 mg).

Several studies have confirmed the importance of GMP in assessing physical dough properties. A high correlation has been already observed between amount of GMP and SDS-sedimentation volume and dough strength according to Moonen et al. (1982), as well as between its concentration and the resistance to extension of wheat dough as stated by MacRitchie & Lafiandra, (1997). A strong relationship was also observed between GMP particle size, i.e. the size of the glutenin particles forming the GMP and that consist of HMW

and LMW subunits, and dough development time (Don et al., 2003a; Don et al., 2003b). Furthermore, Sapirstein & Suchy, (1999) pointed out a strong correlation of gel height after centrifugation and loaf volume of flour samples and ground whole wheat samples ($R2$ = 0.89 and 0.95, respectively).

However, only a few of the available studies enquired into the correlation of GMP and loaf volume and reported that they are closely related to each other (Thanhaeuser et al., 2014; Weegels et al., 1996).

Moreover, this relationship between GMP content and loaf volume should be investigated while considering the effect genotype and the environmental conditions. As reported by Johansson et al., 1999; Zhu and Khan, 2001 the genotype, environment, and their interaction influence baking quality in wheat and this variation in baking quality is related to variation in gluten protein polymer structure. Zhu & Khan, (2001) pointed out that environmental factors might modify molecular sizes of glutenin polymers by influencing the rate of protein synthesis and incorporation of HMW and LMW subunits in forming glutenin polymers which are highly associated with the baking performances. Moreover, genotype has a pivotal influence on the assessment of the quality characteristics, because wheat varieties differed in degree of responsiveness to the environment. So that, one or two samples belonging to a set of samples taken for a study, that are very responsive to the environmental changes, or the other way around, could mainly reduce the correlation values obtained and lead to incorrect conclusions regarding the environment influence on the quality characteristics (Lukov & McVetty, 1991).

Those previous studies underlined the necessity to take a large set of samples in order to integrate influences of location and variety and eventually offer a realistic idea of the estimation of the relationship between GMP and loaf volume.

Thus, the purpose of this study was to highlight the relationship between loaf volume and GMP from approved wheat varieties on the basis of a multigenotype and multilocation analysis. In order to achieve that, GMP of flours from two set of different winter wheat varieties harvested at 8 different locations in Germany from harvest year 2013 and 2014 were extracted under reducing conditions and quantified using HPLC.

Gluten, as the main fraction of wheat flour proteins consists of monomeric gliadins and polymeric glutenins. The gliadins fraction has been reported to contribute to the viscous properties of wheat flour dough, and the glutenin fraction has long been considered to have a crucial role in dough strength and elasticity. A major part of the glutenin fraction is GMP, which may be defined as the large portion of the glutenin that is insoluble in an aqueous SDS solution. The GMP consists mainly of very large structures of HMW and LMW subunits linked by interchain disulphide bonds and have molecular masses roughly ranging from 5 to 20 million (Don et al., 2003a). Thus, they belong to the largest protein complexes in nature (Wrigley, 1996).

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centrifugation and loaf volume of flour samples and ground whole wheat samples ($R2$ = 0.89 and 0.95, respectively).

However, only a few of the available studies enquired into the correlation of GMP and loaf volume and reported that they are closely related to each other (Weegels et al., 1996; Thanhaeuser et al., 2014).

Moreover, this relationship between GMP content and loaf volume should be investigated while considering the effect genotype and the environmental conditions. As reported by Johansson et al., 1999; Zhu and Khan, 2001 the genotype, environment, and their interaction influence baking quality in wheat and this variation in baking quality is related to variation in gluten protein polymer structure. Zhu & Khan, (2001) pointed out that environmental factors might modify molecular sizes of glutenin polymers by influencing the rate of protein synthesis and incorporation of HMW and LMW subunits in forming glutenin polymers which are highly associated with the baking performances. Moreover, genotype has a pivotal influence on the assessment of the quality characteristics, because wheat varieties differed in degree of responsiveness to the environment. So that, one or two samples belonging to a set of samples taken for a study, that are very responsive to the environmental changes, or the other way around, could mainly reduce the correlation values obtained and lead to incorrect conclusions regarding the environment influence on the quality characteristics (Lukov & McVetty, 1991).

Those previous studies underlined the necessity to take a large set of samples in order to integrate influences of location and variety and eventually offer a realistic idea of the estimation of the relationship between GMP and loaf volume.

Thus, the purpose of this study was to highlight the relationship between loaf volume and GMP from approved wheat varieties on the basis of a multigenotype and multilocation analysis. In order to achieve that, GMP of flours from two set of different winter wheat varieties harvested at 8 different locations in Germany from harvest year 2013 and 2014 were extracted under reducing conditions and quantified using HPLC.

II. Article 2

Relationship between glutenin macro polymer and bread wheat baking quality on the basis of multilocation and multigenotype analysis.

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Abstract

Two set of different winter wheat varieties from two harvest years, grown at eight locations in Germany were investigated. The study was conducted to highlight the effect of genotype and environment on the glutenin macropolymer (GMP), high molecular weight (HMW), low molecular weight (LMW) concentrations and the LMW/HMW ratio, as well as to predict the loaf volume by means of the GMP fractions on the basis of a multigenotype and multilocation analysis. GMP fractions were extracted under reducing conditions and quantitated by HPLC. The results obtained demonstrated that, GMP fractions of wheat samples from the harvest year 2013 were mainly influenced by genotype, while GMP fractions of wheat samples from 2014 were affected by location. The relationship between loaf volume and GMP fractions changed significantly with location and genotype for wheat samples from 2013, however wheat samples from 2014 didn't represent a significant correlation between loaf volume and GMP fractions. Furthermore, this relationship does not reach the correlation values obtained between sedimentation value, protein content and loaf volume. This highlights that GMP fractions concentrations cannot predict the loaf volume without taking into account the effect of genotype and location, besides the composition of HMW which also impacts the GMP concentration and the loaf volume.

Keywords: Gluten macro polymer (GMP), loaf volume, location, genotype.

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important worldwide arable crops and is generally processed into a multitude of food products such as bread, pasta and other different types of baked products due to the special rheological properties of wheat proteins (Lagrain et al., 2010). This unique characteristic of wheat proteins is also responsible for the proliferation of bread varieties and all other fermented products, which derives principally from this ability to form a gluten network, which makes the dough able to trap gas bubbles during fermentation and baking, leading to specific volume, and sensory qualities quite different from other baked products (Cauvain, 2003; Mueller et al., 2016).

Gluten, as the major wheat protein, is composed of gliadins and glutenins. A major part of glutenin, with the highest molecular weight, is glutenin macropolymer (GMP) which is unextractable in sodium dodecyl sulphate (SDS) solution (Graveland et al., 1982; Weegels et al., 1996a; Don et al., 2003a). GMP is also known as gel protein, since it forms a gel like layer on top of the starch pellet when SDS extracts are centrifuged (Graveland et al., 1979).

GMP can be isolated by dissolving the SDS unextractable glutenin subfractions under reducing conditions (i.e, interchain disulfide bonds are broken) and subsequently, quantitated using gel permeation HPLC (Cornec et al., 1994; Cinco-Moroyoqui & MacRitchie, 2008). Several studies have confirmed the importance of GMP in assessing physical dough properties. A high correlation has been already observed between amount of GMP and SDS-sedimentation volume and dough strength according to Moonen et al. (1982), as well as between its concentration and the resistance to extension of wheat dough as stated by MacRitchie & Lafiandra, (1997). A strong relationship was also observed between GMP particle size, i.e. the size of the glutenin particles forming the GMP and that consist of high molecular weight (HMW) and low molecular weight (LMW) subunits, and dough development time (Don et al., 2003a; Don et al., 2003b). Furthermore, Sapirstein & Suchy, (1999) pointed out a strong correlation of gel height after centrifugation and loaf volume of flour samples and ground whole wheat samples ($R2$ = 0.89 and 0.95, respectively).

Nevertheless, a few of the available studies enquired into the correlation of GMP and loaf volume and reported that they are closely related to each other (Weegels et al., 1996b)Thanhaeuser et al., 2014).

In addition to that, it is well known that genotype, environment, and their interaction influence baking quality in wheat and that this variation in baking quality is related to variation in gluten protein polymer structure (Johansson et al., 1999; Zhu & Khan, 2001). Zhu & Khan, (2001) pointed out that environmental factors might modify molecular sizes of glutenin polymers by influencing the rate of protein synthesis and incorporation of HMW and LMW subunits in forming glutenin polymers which are highly associated with the baking performances.

Moreover, the nature of wheat varieties has a pivotal influence on the assessment of the quality characteristics, because they differed in degree of responsiveness to the environment. So that, one or two samples belonging to a set of samples taken for a study, that are very responsive to the environmental changes, or the other way around, could mainly reduce the correlation values obtained and lead to incorrect conclusions regarding the environment influence on the quality characteristics (Lukov & McVetty, 1991). Those previous studies underlined the necessity to take a large set of samples in order to integrate influences of location and variety and eventually offer a realistic idea of the estimation of the relationship between GMP and loaf volume.

Thus, the aim of this study was to highlight the relationship between loaf volume and GMP from approved wheat varieties for predicting wheat quality on the basis of a multigenotype and multilocation analysis. In order to achieve that, GMP of flours from two set of different winter wheat varieties harvested at 8 different locations in Germany from harvest year 2013 and 2014 (with one reference variety harvested at 8 different locations in Germany and over the two harvest years) were extracted under reducing conditions and quantified using HPLC. Finally, correlations between loaf volume and GMP concentration were demonstrated as well as the influences of variety and location on the GMP concentration.

2. Material and methods

2.1.Plant material

The samples used in this study are: 15 different soft winter wheat varieties from the harvest year 2013 and 11 different soft winter wheat varieties from the harvest year 2014, each wheat variety is cultivated at eight different locations in Germany that represent the different soil and climate conditions given. The material thus consisted of 120 and 88 wheat flour samples from the harvest years 2013 and 2014, respectively. The wheat varieties are classified according to the German Descriptive Variety List in three groups: Elite wheat (E): n = 4 (2013), n = 2 (2014), Quality wheat (A): n = 8 (2013), n = 1 (2014), and Bread wheat (B): n =

3 (2013), n = 8 (2014). Selected varieties are representatives of the commercial varieties that are registered in the German Descriptive Variety List which classify varieties according to their baking quality as part of the registration process. E-class (elite) wheats have the highest quality, followed by A-class (quality), B-class (bread making) and C-class (not useable for baking) wheat with the lowest quality.

A Buehler laboratory mill MLU 202 was used to mill the wheat grains. Flour type 550 was produced with approximatively 0.60 % ash content, determined according to ICC Standards Methods (ICC 104/1), as recommended by Arbeitsgemeinschaft Getreideforschung, (2016).

2.2.Standard methods

Protein content was determined using Near Infrared Transmission spectroscopy (NITs) with Infratec™ 1241 (Foss GMbH, Rellingen, Germany), according to DIN EN 15948:2012 (protein content % expressed on a dry matter basis). Zeleny Sedimentation value was determined according to ICC 116/1, after preparing the flour samples according to ICC 118.

2.3.Rapid Mix Test

The Rapid Mix Test, as a standard baking method, was carried out according to the method of Pelshenke et al. (2007) to produce bread rolls in order to measure the loaf volume. Before the dough is prepared, the moisture content was determined with the NIT Spectroscopy of the whole grain with Infratec™ 1241(Foss GMbH, Rellingen, Germany) according to the DIN EN 15948:2012, the falling number according to Hagberg-Perten DIN EN ISO 3093:2009, and finally the water absorption (ml/100g) determined using the Farinograph (Brabender GmbH and Co KG, Duisburg, Germany) according to ICC 115/1.

2.4.Extraction and quantitation of GMP

GMP of flours were extracted and quantitated by means of HPLC following the procedure described by Thanhaeuser et al. (2014).

Different chemicals were required for the extraction of GMP: SDS Pellets ≥ 99,5% Rotiphorese-Grade, Hydrochloric acid fuming (HCl) ROTIPURAN® 37%, p.a., and 1,4-Dithiothreitol ≥ 99% (DTT), p.a., were from Carl Roth GmbH + Co KG (Karlsruhe, Germany), di-Sodium hydrogen phosphate dehydrate (NaH2PO4), Potassium dihydrogen phosphate (HKNaPO4) and 1-Propanol for analysis EMSURE® ACS,Reag. Ph Eur. ≤ 100% were purchased from Merck KGaA (Darmstadt, Germany).

100 mg of flour was weighted into a Pyrex tube (capacity: 12 mL), then extracted twice at room temperature with solvent A (1% (w/v) SDS and 0.05 mol/L NaH2PO4, (pH 6.9); the pH value was adjusted with HCl (32%, w/w)), and twice at 60° C with solvent B (50% (v/v) 1-propanol, 0.05 mol/L HKNaPO4 (pH 7.5) and 1% (w/v) DTT) under nitrogen. Each extraction step was initiated with vortexing for 2 min at room temperature and followed by magnetic stirring for 30 min (at room temperature when solvent A was used, and at 60° C when solvent B was used) and continued by a centrifugation of the suspension for 25 min at 4600 rpm/min at room temperature (Heraeus Megafuge 1.0, Thermo Fisher Scientific, Dreieich, Germany). The supernatants were transferred to a volumetric flask (capacity: 2 mL) and diluted to 2 mL with solvent B. The extracts were filtered through a 0.45µm filter, transferred into the HPLC vials.

A Kontron HPLC instrument (Kontron Instrument, Roßdorf, Germany) fitted with a Yarra 3u SEC-3000 column (Phenomenex, Aschaffenburg, Germany) was used to analyse the extracts under the following conditions: The column temperature was the room temperature, the injection volume was 20 µL, the eluent was solvent A at a flow rate of 0.3 mL/min, and detection was made by measuring the UV absorbance at 210 nm by means of a Kontron 332 UV-Vis-Detektor. The integration of the obtained areas was performed using Chromgate software (Knauer, Berlin, Germany).

As a reference material for the calibration, gliadin reference from the Prolamin Workin Group (PWG gliadin) (van Eckert et al., 2006) was used.

The extractions and measurements were carried out for each flour sample in duplicate.

2.5.Statistical analysis

Statistical analysis (Pearson correlation coefficient and multiple regression analysis) were performed using SigmaPlot for windows version 11 (Systat Software Inc., San Jose, USA).

The Kruskal-Wallis test as a nonparametric (distribution free) test was chosen to determine if quality parameters, loaf volume and GMP fractions differed significantly according to wheat quality classes, location and genotype. This test didn't require a normal distribution of dependent variables. Evaluation of the data was carried out using median values and standard deviations, Values with $p \leq 0.001$ were considered significantly different. Subsequently Dunn's Test as Post Hoc Test was run when a statistically significant difference in group medians were observed to confirm where the differences occurred between groups (Values

with $p < 0.05$ were considered significantly different). Dunn's Test was used because it is the only Post Hoc Test available after a Kruskal Wallis Test when the sample sizes are not equal. Correlation coefficients among all parameters considered were evaluated based on the whole set of data, the means of genotypes belonging to each quality class, the means of all locations for each genotype and the means of all genotypes in the individual location. Therefore, a linear Pearson correlation was carried out.

3. Results and Discussion

The analysis of the wheat samples used for the study revealed a large variation in the physico-chemical parameters measured: The crude protein content ranges from 11.1 to 16.3 % DM in 2013, however it appears relatively lower in 2014 (10.4-15.6 % DM), the sedimentation value from 19 to 75 ml (21-75 ml (2014)) and the loaf volume from 480 to 783 ml/100 g (460-800 ml/100 g (2014)).

3.1.Concentrations of GMP, HMW, LMW subunits and LMW/HMW ratios

An extraction of GMP under reducing conditions and quantification by means of HPLC following the procedure described by Thanhaeuser et al. (2014), enabling the quantitation of the total GMP amount, as well as HMW and LMW subunits (Fig. 1). This quantitation was preceded by a calibration of the HPLC-column by analysing a mixture of proteins with different molecular weight. The total GMP peak contains two main peaks representing HMW and LMW subunits, and occurring at 7.4 min and 8.4 min, respectively, which is similar to the results obtained by Cinco-Moroyoqui and MacRitchie, (2008) and Thanhaeuser et al. (2014).The small peak appearing at 9.4 min corresponds to albumins and globulins, which was fully integrated in this study with the peak of LMW.

The GMP concentrations of flour samples obtained for the eight locations from the harvest year 2013 and 2014 ranged from 1.14 mg/g (Diantha (wheat B-class), location 4) to 20.85 mg/g (Kompass (wheat A-class), location 2) to, and from 0.76 mg/g (Ponticus (wheat E-class) location 5 and Partner (wheat B-class), location 3) to 9.18 mg/g (Produzent (wheat B-class), location 1), respectively (data not shown. Flour samples from the harvest year 2013 showed higher GMP concentrations in comparison with flour samples from 2014. The low overall protein content in flour samples from 2014 can be explained by the notably high grain yields, which are known to decrease the protein/starch ratio in the kernels, which is a consequence of the heat effect during the grain filling according to the studies of Ciaffi et al., (1996); Naeem

et al., (2002); and Don et al., (2005b). This heat effect is attributed to the environmental conditions characterising the harvest year 2014 in Germany (Deutscher Wetterdienst, 2014). Increased temperature during grain filling induces disturbances by the GMP particle formation. Heat stress accelerates physiological and biochemical reactions in grains, which accelerates the rate of grain dehydration, and subsequently shortens the time of grain development (grain filling rate is accelerated) (Cairns et al., 2012). This situation slows the biosynthesis of glutenin as reported also by DuPont and Altenbach, (2003), leading to a decrease in the GMP concentration with an increase of glutenin particles. Higher temperatures and dehydration induce a more rapid and an increased disulphide bonds formation at the time of polymerisation and assembly into larger insoluble glutenin particles, as well as a fusion of glutenin particles, which cause an hyperaggregation and formation of larger particles (Carceller and Aussenac, 2001; Don et al., 2005b). This outcome is supported by the GMP concentrations of Julius, the only one reference variety harvested over the two harvest years. We obtained a mean concentration of 3.9 mg/g for 2013, and a lower concentration for 2014 of 3.6 mg/g.

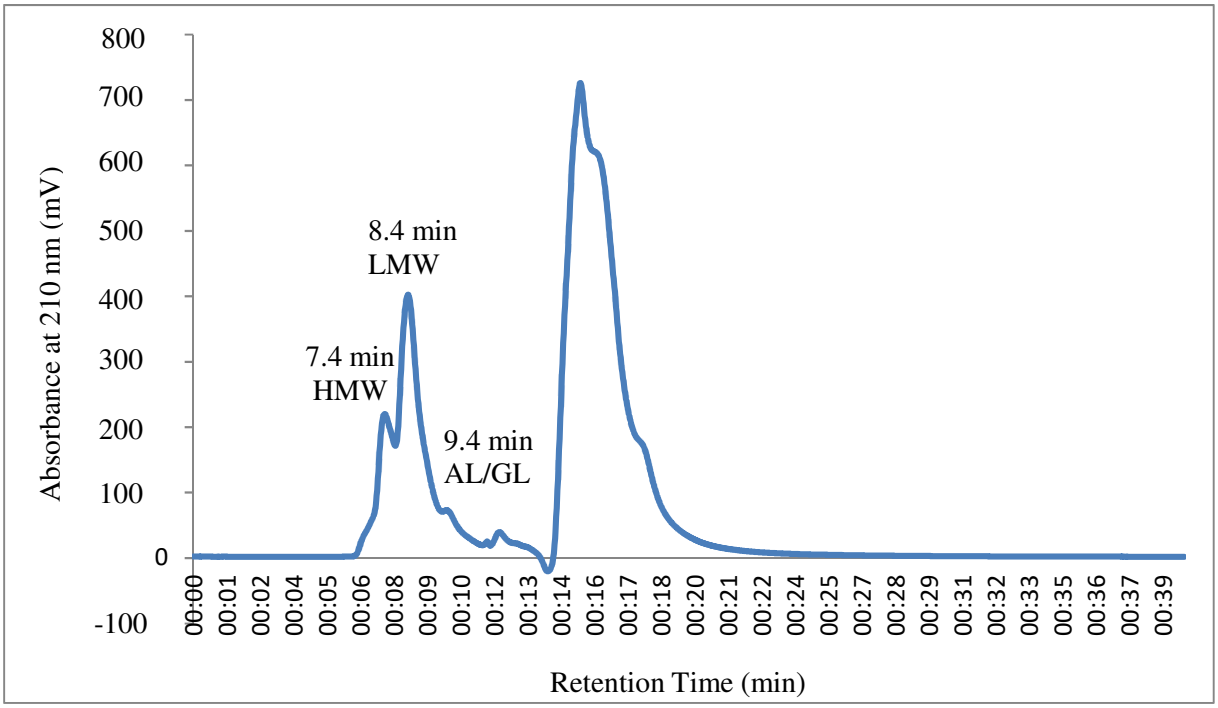


Figure 1: HPLC profil of separation of GMP fractions, showing HMW, LMW and albumins globulins of Axioma (wheat E-class) from location 8 and the harvest year 2013

The results obtained regarding the GMP concentrations are sometimes for some cultivars and locations lower than those got by Thanhaeuser et al. (2014), who achieved GMP

concentrations ranging from 8.3 to 18.4 mg of protein/g of flour. This can be attributed to the effect of cultivar and environment on GMP, the effect that will be highlighted in this study. Furthermore, differences in plant material preparation between our study and the study of Thanhaeuser et al. (2014), who used for example for the milling a Quadrumat junior Mill from Brabender at variance with the Buehler laboratory mill MLU 202 that we used to mill the wheat grains, can also have an influence. The milling process affects strongly wheat flour composition and wheat quality characteristics as reported by El-Porai et al. (2013).

HMW concentrations of wheat samples from the harvest years 2013 and 2014 ranged from 0.05 mg/g (Diantha (wheat B-class), location 1) to 4.21 mg/g (Axioma (wheat E-class), location 1) and from 0.19 mg/g (Ponticus (wheat E-class), location 5) to 1.99 mg/g (Produzent (wheat B-class), location 7), respectively. Don et al. (2004) showed the importance of HMW in determining the GMP concentration and pointed out that the amount of GMP increased with the increasing amount of HMW. However, the LMW/HMW ratios calculated in this study provide a different statement. Even if we obtained high GMP concentrations for some wheat varieties, they displayed higher LMW/HMW ratios, as can be seen for the wheat samples RGT Reform (wheat A-class, location 3) and Kobold (wheat E-class, location 3) from the harvest year 2013 (GMP concentrations: 10.86 mg/g and 18.18 mg/g, respectively; LMW/HMW ratio: 8.05 and 14.37, respectively). This agrees with findings of Southan and MacRitchie (1999), and Don et al. (2005a) that in addition to quantity, the presence of specific HMW play a role in determining the quantity of GMP.

The LMW/HMW ratio ranged in our study for wheat samples from 2013 from 2.22 (Axioma (wheat E-class), location 1) to 6.81 (Dichter (wheat A-class), location 2), except in the case of Diantha (wheat B-class, location 1 and 3) which showed very low HMW concentrations (0.05 mg/g in location 1 and 0.16 mg/g in location 3), and subsequently lead to very high LMW/HMW ratios (20.28 in location 1 and 10.56 in location 3). Regarding wheat samples from the harvest 2014, this ratio varies between 3.15 (Produzent (wheat B-class), location 7) and 6.44 (Alexander (wheat B-class), location 6). The cultivar Julius (wheat A-class) harvested over the two years, displayed LMW/HMW ratios ranging from 3.44 to 5.18 in 2013, that are increasing in most locations in 2014, for example in 2013 the ratios for locations 3 and 5 were: 4.03 and 3.44, respectively, and increased in 2014 to achieve 4.29 and 4.84, respectively. This phenomenon agrees with the previous study of Don et al. (2005b), who reported that the LMW/HMW ratio of GMP is increased by heat stress. The same cultivar Julius was a question of interest in the study of Thanhaeuser et al. (2014) (from one

location and the harvest year 2010), in which the LMW/HMW ratio was lower than the ratio obtained in our study over the 8 locations and the 2 harvest years. The difference between our outcome and the results of this study may be the effect of the environmental conditions (harvest year and location of cultivation) and the used milling device. For that reason, it is necessary to investigate the effect of genotype and location, as well as of the quality class on the GMP, HMW, LMW concentrations and LMW/HMW ratio.

3.2. Effect of wheat quality class, location and genotype on indirect parameters, loaf volume and GMP fractions.

In order to emphasize the effect of quality class, location and genotype on indirect parameters, loaf volume and GMP fractions, table 1 presents the wheat quality class (E, A, and B) median values, location median values and genotype median values of wheat samples over the 8 locations and the two harvest years 2013 and 2014 for crude protein content, sedimentation value, loaf volume and GMP fractions that means GMP, HMW, LMW concentrations and finally LMW/HMW ratio. Subsequently the significant different pairs ($p < 0.05$) resulting from the pairwise comparisons (Dunn's Test) of protein content, sedimentation level, loaf volume, GMP, HMW, LMW concentrations and LMW/HMW ratio according to wheat quality class, location and genotype were determined.

In harvest year 2013, wheat quality class was observed to be statistically significant source of variation ($p \leq 0.001$) for all considered parameters. Samples of wheat E-class showed the highest median for all investigated parameters, followed by samples of wheat A-class and finally samples of wheat B-class, except LMW/HMW ratio which showed the opposite direction (E-class < A-class < B class). We can also remark that in figure 2, which displayed protein content, GMP concentration and LMW/HMW ratio of wheat samples belonging to wheat quality classes E, A and B and harvest year 2013. The variation between wheat quality classes lies in crude protein content, sedimentation value and loaf volume between all quality classes, however concerning GMP, HMW, LMW and LMW/HMW ratio, this significant difference is observed only between wheat E-class and B-class, and between E-class and A-class (Table 2). This variation between wheat quality classes is considered as an expected result, because the German wheat classification system allocates samples to a certain quality group on the basis of many quality traits (among them we have crude protein content, sedimentation and loaf volume), in such a way that E-class wheat has the highest quality, followed by A-class, B-class and C-class wheat with the lowest quality (not useable for baking and hence not investigated in our study) (Laidig et al., 2016). Moreover, GMP

fraction is associated with protein content which explained the results obtained regarding GMP, HMW, and LMW concentrations for each wheat quality class (Spiertz et al., 2006).
In contrast, wheat samples from 2014 didn't present any significant difference between wheat quality classes for GMP, HMW, LMW concentrations and LMW/HMW. This might be the consequence of heat effect on GMP concentration (DuPont and Altenbach, 2003), and afterwards on GMP fractions, which didn't allow a differentiation between wheat quality classes on the basis of these parameters. In addition to that, a significant distinction of wheat quality classes based on protein content, sedimentation value and loaf volume was exclusively possible between wheat E- class and B- class for protein content and loaf volume, and between E- class and B- class, A- class and B- class for sedimentation value (Table 2).
A significant distinction between wheat quality classes based on GMP fractions and LMW/HMW proves possible with wheat samples belonging to the harvest year 2013. Nevertheless, wheat samples from the harvest year 2014 showed the opposite case, which can be attributed to the impact of high temperature on wheat grain.

Another factor was considered to highlight its effect on the quality parameters considered, which is location of growth. For wheat samples belonging to the harvest year 2013, differences were found among locations for protein content, sedimentation value and LMW/HMW ratio (Table 1). Location 1 showed the highest median value of protein content (13.8 % DM) and differed significantly from locations 4, 5, and 6 (data not shown), while location 7 has the highest sedimentation value (61 ml) and displayed a significant difference with locations 4 and 5. Location 3 has the highest values of GMP, HMW, LMW concentrations and a low LMW/HMW ratio, but no significant difference in GMP, HMW and LMW concentrations was obtained between locations, indicating that these parameters are not influenced by location in this harvest year.

Table 1: Wheat quality class (E, A, B) median values, location median values and genotype median values of wheat samples over the 8 locations and the two harvest years 2013 and 2014 for crude protein content, sedimentation value, loaf volume, GMP, HMW, LMW concentrations and LMW/HMW ratio

Harvest year		Crude protein content (% DM)	Sediment-ation value (ml)	Loaf volume (ml/ 100mg)	GMP (mg/g)	HMW (mg/g)	LMW (mg/g)	LMW/HMW ratio
	Wheat quality classes							
2013	E	13.8	66	685	9.39	2.21	7.06	3.30
	A	13.0	49	640	5.58	1.16	4.44	3.77
	B	12,4	27	570	3.87	0.74	2.94	4.02
	H	25.7	58	62	30.45	33.62	28.70	17.06
	p	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
2014	E	14.2	71	674	4.30	0.80	3.66	4.65
	A	12.8	63	604	3.74	0.72	3.16	4.38
	B	12.1	36	570	2.99	0.58	2.59	4.49
	H	35.1	45	36	3.79	3.95	4.00	0.39
	p	≤0.001	≤0.001	≤0.001	n.s[(a)]	n.s	n.s	n.s
	Location							
2013	1	13.8	59	670	6.56	1.64	4.69	3.12
	2	13.1	52	622	5.80	1.28	4.46	3.73
	3	12.9	56	628	7.88	1.98	6.01	3.13
	4	11.8	41	611	5.42	1.14	4.53	3.96
	5	12.4	41	648	4.92	1.14	3.88	3.44
	6	12.5	42	655	6.22	1.26	5.11	4.15
	7	13.6	61	665	7.45	1.80	5.96	3.63
	8	13.7	54	683	6.19	1.06	5.16	4.02
	H	50.8	28	16	13.04	13.84	12.02	34.98
	p	≤0.001	≤0.001	0.029	n.s	n.s	n.s	≤0.001
2014	1	12.1	44	570	6.34	1.28	5.14	3.92
	2	12.8	47	599	3.65	0.65	3.16	5.24
	3	12.2	38	574	3.38	0.67	2.87	4.45
	4	10.9	30	554	2.56	0.48	2.18	4.43
	5	12.9	51	582	1.24	0.30	1.08	4.18
	6	12.1	43	582	3.99	0.73	3.39	4.60
	7	11.2	33	570	3.60	0.74	3.02	4.20
	8	12.7	46	623	3.88	0.65	3.30	5.13
	H	38.1	17	19	40.68	38.95	41.58	38.85
	p	≤0.001	0.017	0.008	≤0.001	≤0.001	≤0.001	≤0.001

	Genotype							
2013	Julius	12.2	50	598	3.39	0.61	2.94	4.14
	Kompass	13.0	49	649	10.21	2.04	8.48	4.09
	Dichter	13.3	40	630	3.57	0.71	2.71	3.77
	Attraktion	13.0	59	643	4.96	1.00	4.09	3.66
	Diantha	12.4	23	500	1.43	0.27	1.25	5.13
	RGT Reform	12.9	51	649	7.33	1.57	5.92	3.67
	KWS Magic	13.0	48	663	5.55	1.23	4.48	3.70
	KWS Loft	12.6	43	603	8.42	1.76	6.67	3.86
	KWS Montana	13.6	62	679	8.20	1.98	6.15	3.00
	Spontan	13.7	61	660	5.12	1.13	4.09	3.42
	Axioma	15.2	74	700	13.67	3.44	9.71	3.21
	Jonny	12.3	27	564	3.68	0.73	2.92	3.93
	Franz	12.2	48	635	6.39	1.34	5.21	3.66
	Bernstein	13.5	53	661	10.31	2.21	7.77	3.74
	Kobold	13.1	61	683	6.30	1.48	4.80	3.64
	H	43.9	75	73	92.46	87.82	94.05	38.60
	p	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
2014	Julius	12.8	63	604	3.74	0.72	3.16	4.38
	Produzent	12.3	51	564	4.46	0.83	3.78	4.14
	KWS Salix	11.8	35	585	2.39	0.46	2.10	4.34
	Bonanza	12.0	44	520	3.57	0.62	3.11	4.21
	Gustav	12.5	47	576	4.42	0.75	3.81	4.55
	Benchmark	11.8	30	570	2.34	0.43	2.06	4.67
	Faustus	12.1	32	569	2.55	0.47	2.24	4.71
	Ponticus	13.9	68	636	4.06	0.77	3.41	4.44
	Helmond	14.4	72	735	6.29	1.09	5.33	4.87
	Alexander	11.8	32	599	2.22	0.37	2.00	5.22
	Partner	12.3	37	563	3.07	0.53	2.69	4.65
	H	41.0	64	51	23.55	23.23	23.34	13.08
	p	≤0.001	≤0.001	≤0.001	0.009	0.010	0.010	n.s

[(a)] $p > 0.05$, not significant (n.s)

Table 2: Significant different pairs ($p < 0.05$) resulting from Dunn's Test of protein content, sedimentation level, loaf volume, GMP, HMW, LMW concentrations and LMW/HMW ratio according to wheat quality classes.

Harvest year	Parameters	Comparison	P	P<0,050
2013	Crude protein content	E vs B	<0.001	Yes
		E vs A	0.002	Yes
		A vs B	0.037	Yes
	Sedimentation value	E vs B	<0.001	Yes
		E vs A	<0.001	Yes
		A vs B	<0.001	Yes
	Loaf volume	E vs B	<0.001	Yes
		E vs A	<0.001	Yes
		A vs B	<0.001	Yes
	GMP concentration	E vs B	<0.001	Yes
		E vs A	<0.001	Yes
		A vs B	n.s[(a)]	No
	HMW concentration	E vs B	<0.001	Yes
		E vs A	<0.001	Yes
		A vs B	n.s	No
	LMW concentration	E vs B	<0.001	Yes
		E vs A	<0.001	Yes
		A vs B	n.s	No
	LMW/HMW ratio	B vs E	<0.001	Yes
		B vs A	n.s	No
		A vs E	0.005	Yes
2014	Crude protein content	E vs B	<0.001	Yes
		E vs A	n.s	No
		A vs B	n.s	No
	Sedimentation value	E vs B	<0.001	Yes
		E vs A	n.s	No
		A vs B	0.001	Yes
	Loaf volume	E vs B	<0.001	Yes
		E vs A	n.s	No
		A vs B	n.s	No

[(a)] $p > 0.05$, not significant (n.s)

Concerning wheat samples of harvest year 2014, statistical analysis showed significant differences ($p \leq 0.001$) in protein content, GMP, HMW, LMW concentrations and LMW/HMW ratio among the growing locations, indicating that the location affected these parameters. Throughout the eight locations, the highest concentration of GMP, HMW, LMW (6.34 mg/g, 1.28 mg/g, and 5.14 mg/g) and the lowest value of LMW/HMW ratio (3.92 mg/g) was obtained at location 1. This location is significantly different from location 4 and 5 for GMP, HMW and LMW, and from location 2, 6 and 8 for LMW/HMW ratio. This outcome is in accordance with the conclusion of Wieser and Zimmermann (2000), Jin et al. 2011, Dhaka and Khatkar (2014) who suggested that HMW plays a positive role in dough rheological properties by contributing more to dough strength than the LMW and illustrated the dependence of baking quality on LMW/HMW specifying that higher HMW concentration in comparison with LMW concentration lead to a higher baking quality. The correlation between dough strengthening and LMW/HMW might be linked to the fact that the HMW amount helps in forming the backbone of glutenin network (Dhaka and Khatkar, 2014).

Location 5 displayed a significant difference with location 4 regarding sedimentation value and with location 7 regarding protein content, GMP, HMW and LMW concentrations.

The relationship between GMP concentrations of all varieties belonging to the eight locations over the two harvest years 2013 and 2014 (results not shown) approves the previous obtained outcome, so that the coefficients of correlation achieved between the different locations for wheat samples belonging to the harvest year 2013 were very high and varied from 0.72 (between location 3 and 5) to 0.98 (between location 1 and 7) indicating weak fluctuations between the different locations regarding the GMP concentration. In contrast to that, the coefficients of correlation obtained between the eight locations for wheat samples of the harvest year 2014 revealed in lot of cases the influence of the specifications of each location on the GMP concentrations manifested by low correlations.

As described in table 1, genotype was statistically significant source of variation for all the quality parameters considered ($p \leq 0.001$) for wheat samples belonging to the harvest year 2013. Among the genotypes studied, Axioma showed the highest protein content (15.2 % DM), sedimentation value (74 ml), loaf volume (700 ml/100g), GMP (13.67 mg/g), HMW (3.44 mg/g) and LMW (9.71 mg/g) concentrations and approximately the lowest LMW/HMW ratio (3.21), and differed significantly from Diantha, Dichter, Jonny, Franz, Julius and KWS Loft (Table 2). While Diantha had the lowest values of sedimentation value (23 ml), loaf volume (500 ml/100g), GMP (1.43 mg/g), HMW (0.27 mg/g), LMW (1.25 mg/g) and the highest value of LMW/HMW (5.13), and differed from KWS Montana, Kobold, Bernstein,

Spontan and Attraktion for sedimentation value and loaf volume, and from Kompass, KWS Montana, Bernstein, KWS Loft and RGT Reform for GMP, HMW, LMW concentrations.

About wheat samples belonging to the harvest year 2014, there was no significant difference in GMP, HMW, LMW concentrations and LMW/HMW ratio between the genotypes (Table1). However, the protein content, sedimentation value and loaf volume, showed a significant difference among genotypes, suggesting that a distinction between genotypes can be achieved on the basis of these three parameters. Genotype Helmond showed the highest values of protein content (14.4 % DM), sedimentation value (72 ml), loaf volume (735 ml/100g), GMP (6.29 mg/g), HMW (1.09 mg/g), and LMW (5.33 mg/g) and is followed by Ponticus regarding protein content (13.9 % DM), sedimentation value (72 ml) and loaf volume (636 ml/100g). Helmond is significantly different for those three parameters from Benchmark and Faustus.

In summary, wheat quality class, location and genotype are statistically significant sources of variation as determined by the results obtained for the quality parameters considered, in such a way that for wheat samples from harvest year 2013, the distinction between quality classes and genotypes is acquired with all parameters examined, that means protein content, sedimentation value, loaf volume, GMP, HMW, LMW concentrations and LMW/HMW ratio, while the distinction between locations is possible only on the basis of protein content, sedimentation value and the ratio LMW/HMW. Moreover, from table 1 we can conclude that location represented the largest source of variation for protein content ($H = 50.8$, $p \leq 0.001$) in comparison with quality class and genotype ($H = 25.7$, $p \leq 0.001$ and $H = 43.9$, $p \leq 0.001$, respectively), when genotype affected more the other measured parameters, sedimentation value, loaf volume, GMP, HMW, LMW concentrations and LMW/HMW ratio. This outcome is confirmed by the study of Johansson et al. (2001), who reported that protein concentration is determined both genetically and environmentally. In addition to that, Zhu and Khan (2001) investigated six genotypes of hard red spring wheat, grown at seven environments in North Dakota during 1998 and demonstrated that gluten macro polymer was controlled more by genetic than environmental factors, which was also highlighted in our present study.

Regarding wheat samples from the harvest year 2014, protein content is also affected by genotype and environment but it turn out that it's more influenced by genotype ($H = 40.9$, $p \leq 0.001$) than location ($H = 38.1$, $p \leq 0.001$) or quality class ($H = 35.1$, $p \leq 0.001$).

This difference seen between wheat samples from 2013 and 2014 regarding protein content may be attributed to the high temperatures, affecting wheat grain in terms of various

physiological and biochemical processes, and which depends on the responsiveness and sensitivity of each genotype to the environment (Iqbal et al., 2017; de Leonardis et al., 2015).

3.3.Relationship between GMP fractions, indirect parameters and loaf volume

The correlation between loaf volume and, protein content, sedimentation value and GMP fractions for wheat samples from harvest year 2013 and harvest year 2014 is summarized in table 3.

Wheat flour samples from 2013 showed a good correlation between protein content, sedimentation value and loaf volume ($r = 0.65$, $p < 0.001$; $r = 0.78$, $p < 0.001$, respectively) confirming previous studies (Hruskova et al., 2001; Perez Borla et al., 2004; Różyło and Laskowski, 2011). Meanwhile, the protein content of wheat flour samples from 2014 is more closely related to the loaf volume ($r = 0.81$, $p < 0.001$) by comparison with the correlation between sedimentation value and loaf volume ($r = 0.64$, $p < 0.001$).

In 2013, all measured parameters (GMP, HMW, LMW concentrations), except LMW/HMW showed positive correlation with loaf volume, while wheat samples from 2014 didn't show any significant relation to loaf volume. HMW concentration showed the highest correlation ($r = 0.55$, $p < 0.001$) in 2013 followed by GMP ($r = 0.50$, $p < 0.001$). These observations didn't confirm with those obtained by Thanseauser et al. (2014) who obtained a close relationship between GMP fractions and loaf volume (GMP: $r = 0.80$, HMW: $r = 0.77$ and LMW: $r = 0.77$), which is probably linked to the different baking tests applied in the two studies. Thanseauser et al. (2014) used the Microscale Baking Test as a baking test with 10 g of flour carried out according to the method of Selmair and Koehler (2008) with an optimised mixing of each flour to optimum consistency, instead of the Rapid Mix test that we used in our study, which is assumed to have a large impact on the correlation between loaf volume and the GMP fractions. The Rapid Mix Test requires dough kneading of 1 min at a speed of 1400 rpm which might be capable of disintegrating the GMP-Network, in contrast with a gentle kneading which can maintains the GMP-Network, in the case of the Micro Rapid Mix Test with a mixing of 1 min at 1250 rpm. In addition to that, the differences between the two studies in the plant material preparation, like for example milling, impacts the protein content, GMP fractions and subsequently the different correlation values. Moreover, the coefficient of correlation between loaf volume and GMP fractions does not reach the correlation values obtained between sedimentation value, protein content and loaf volume. These relationships might be changed due to different environmental and genotype influences.

Table 3: The relationship between protein content, sedimentation value, GMP fractions and loaf volume for wheat samples from harvest year 2013 and harvest year 2014

Parameters		Crude Protein content (% DM)	Sedimentation value (ml)	GMP (mg/g)	HMW (mg/g)	LMW (mg/g)	LMW/HMW ratio
Flour samples 2013 Loaf volume (ml/100g)	r	0.65	0.78	0.50	0.55	0.48	-0.43
Loaf volume (ml/100g)	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Flour samples 2014 Loaf volume (ml/100g)	r	0.81	0.64	0.19	0.11	0.21	0.31
	p	<0.001	<0.001	n.s[(a)]	n.s	0.045	0.004

[(a)] $p > 0.05$, not significant (n.s)

Therefore, the determination of the relationship between GMP fractions and loaf volume requires considering the effect of quality class, genotype and location on this correlation, especially since we previously highlighted their effect of these quality parameters. Thus, correlations were computed in three different ways: using quality classes means, location means and finally genotype means.

Table 4 displayed the relationship between protein content, sedimentation value, GMP, HMW, LMW concentrations and LMW/HMW ratio and loaf volume of wheat samples belonging to different wheat quality classes (E; A; B) from harvest year 2013 and 2014.

Regardless of the year, wheat samples of E-class showed the highest correlation with loaf volume, followed by wheat A-class and wheat B-class, contrary to the coefficient of correlation obtained between sedimentation value and loaf volume, which attributed the highest correlation to wheat B-class. With reference to the relationship between GMP fractions and loaf volume, the highest coefficient of correlation was assigned to the samples belonging to wheat B-class and obtained with HMW concentration ($r = 0.72$, $p < 0.001$), while samples of wheat E-class and A-class didn't show any significant correlation with ($r = 0.37$, $p = 0.14$; $r = 0.14$, $p = 0.28$, respectively). This outcome demonstrated that quality class doesn't affect the relationship between GMP fractions and loaf volume of soft wheat flour samples.

Table 4: The relationship between protein content, sedimentation value, GMP, HMW, LMW concentrations and LMW/HMW ratio and loaf volume of wheat samples belonging to different wheat quality classes (E, A, B) from harvest year 2013 and 2014.

Harvest year	Wheat quality classes		Crude protein content (% DM)	Sedimentation value (ml)	GMP (mg/g)	HMW (mg/g)	LMW (mg/g)	LMW/HMW ratio
2013	E	r	0.81	0.60	0.29	0.37	0.27	-0.25
		p	<0.001	<0.001	n.s[(a)]	0.009	0.037	n.s
	A	r	0.54	0.42	0.09	0.14	0.08	-0.20
		p	<0.001	<0.001	n.s	n.s	n.s	n.s
	B	r	0.08	0.64	0.71	0.72	0.68	-0.49
		p	n.s	<0.001	<0.001	<0.001	<0.001	<0.001
2014	E	r	0.79	0.63	0.28	0.20	0.30	0.24
		p	<0.001	0.009	n.s	n.s	n.s	n.s
	A	r	0.72	0.66	0.25	0.16	0.27	0.27
		p	n.s	n.s	n.s	n.s	n.s	n.s
	B	r	0.52	0.14	-0.08	-0.16	-0.06	0.46
		p	<0.001	n.s	n.s	n.s	n.s	n.s

[(a)] $p > 0.05$, not significant (n.s)

Table 5 emphasizes the effect of location on the relationship between indirect methods, GMP fractions and loaf volume. The correlation between protein content, sedimentation value and loaf volume of flour samples from eight locations and the harvest year 2013 ranged from 0.41 to 0.78 and from 0.68 to 0.91, respectively. Except location 2, all other locations displayed a positive significant correlation between GMP, HMW, LMW concentrations and ranged as follows: 0.41-0.73, 0.41-0.77, and 0.41-0.71. Location 7 showed the highest correlation between GMP fractions and loaf volume, while location 5 showed the lowest correlation between these parameters. All locations displayed a negative correlation between loaf volume and LMW/HMW ratio, but the highest negative correlation was also obtained in location 7. Moreover the highest correlation achieved with loaf volume fluctuates from GMP fraction to another or to the LMW/HMW ratio depending on each location, for example in location 1 and 3, the highest correlation was with LMW/HMW ratio, and in location 4 and 7 the highest correlation was obtained with HMW concentration.

Table 5: The relationship between protein content, sedimentation value, GMP, HMW, LMW concentrations and LMW/HMW ratio and loaf volume of wheat samples according to each location from harvest year 2013 and 2014

Harvest year	Location		Crude protein content (% DM)	Sedimentation value (ml)	GMP (mg/g)	HMW (mg/g)	LMW (mg/g)	LMW/HMW ratio
2013	1	r	0.54	0.81	0.69	0.66	0.69	-0.78
		p	0.04	0.001	0.005	0.007	0.004	0.001
	2	r	0.73	0.80	0.08	0.20	0.04	-0.38
		p	0.005	0.001	n.s[(a)]	n.s	n.s	n.s
	3	r	0.75	0.88	0.57	0.71	0.51	-0.72
		p	<0.001	<0.001	0.043	0.006	n.s	0.006
	4	r	0.78	0.90	0.71	0.73	0.70	-0.46
		p	<0.001	<0.001	0.003	0.002	0.004	n.s
	5	r	0.41	0.68	0.41	0.41	0.41	-0.03
		p	n.s	0.01	n.s	n.s	n.s	n.s
	6	r	0.63	0.78	0.50	0.58	0.48	-0.59
		p	<0.001	<0.001	n.s	0.024	n.s	0.022
	7	r	0.71	0.84	0.73	0.77	0.71	-0.70
		p	<0.001	<0.001	0.002	0.001	0.003	0.004
	8	r	0.76	0.91	0.56	0.58	0.56	-0.52
		p	<0.001	<0.001	0.029	0.024	0.032	0.047
2014	1	r	0.83	0.55	0.13	0.19	0.12	-0.24
		p	<0.001	n.s	n.s	n.s	n.s	n.s
	2	r	0.74	0.42	0.49	0.41	0.50	0.36
		p	0.01	n.s	n.s	n.s	n.s	n.s
	3	r	0.70	0.62	0.01	-0.12	0.04	0.76
		p	0.02	0.04	n.s	n.s	n.s	0.007
	4	r	0.72	0.72	0.30	0.14	0.34	0.52
		p	0.01	0.01	n.s	n.s	n.s	n.s
	5	r	0.83	0.51	-0.55	-0.61	-0.52	0.54
		p	<0.001	n.s	n.s	0.048	n.s	n.s
	6	r	0.85	0.74	0.56	0.59	0.54	-0.37
		p	<0.001	0.01	n.s	n.s	n.s	n.s
	7	r	0.86	0.83	0.34	0.23	0.37	0.13
		p	<0.001	<0.001	n.s	n.s	n.s	n.s
	8	r	0.91	0.73	0.68	0.55	0.70	0.28
		p	<0.001	0.01	0.022	n.s	0.017	n.s

[(a)] $p > 0.05$, not significant (n.s)

Those findings indicate that the relationship between loaf volume and GMP fractions significantly changed with location, confirming previous study (Zhu and Khan, 2001), which supposed that environment affected the formation of glutenin macro polymers by modifying molecular sizes of glutenin polymers by influencing the rate of protein synthesis and incorporation of HMW and LMW subunits in forming glutenin polymers.

For wheat flour samples from the harvest year 2014, the ranges of the coefficients of correlation between protein content, sedimentation value and loaf volume were: 0.70-0.91 and 0.42-0.83, respectively. Except location 8 which presented the highest relationship between HMW, LMW concentrations and loaf volume, almost no location represents a significant correlation between loaf volume and GMP fractions. This outcome might be explained by the effect of heat on the genotypes present in these locations, which disturbed the biosynthesis of GMP fractions and subsequently gave a non-significant estimation of the relationship between them and loaf volume, as described previously.

Table 6 presented the effect of genotype on the correlation between indirect methods, GMP fractions and loaf volume. Among the genotypes investigated belonging to the harvest 2013, KWS Montana and Bernstein showed the highest correlation between protein content and loaf volume, while Dichter displayed a high correlation between GMP, HMW, LMW and loaf volume, even if it showed no significant correlation between protein content, sedimentation value and loaf volume. Sometimes HMW concentration seems to be the parameter achieving the highest correlation with loaf volume, like in the case of Franz, KWS Montana and Dichter and for other genotypes LMW concentration or GMP showed this high correlation. That indicated that the correlation between GMP fractions and loaf volume varied according to each genotype, as demonstrated by Zhu and Khan, (2001). The ranges of coefficient of correlation between protein content, sedimentation value and loaf volume of wheat flour samples of the harvest 2014 were: 0.54 (Faustus) – 0.97 (Helmond) and 0.28 (Gustav) – 0.89 (Alexander), respectively. However, GMP fractions didn't display a significant relationship with loaf volume, which can be explained by the effect of high temperature, as observed across the same set of data, when we investigated the effect of location, and as well as by the effect of high kneading during the baking test, which can lead to a disaggregation of the GMP-Network, generating no significant correlation between loaf volume and GMP fractions. Moreover, GMP, HMW and LMW concentration are not the only factors affecting the relationship with loaf volume, the composition of HMW impacts also the GMP concentration and the loaf volume (Shewry et al., 1992).

Table 6: The relationship between protein content, sedimentation value, GMP, HMW, LMW concentrations and LMW/HMW ratio and loaf volume of wheat samples according to each genotype over all locations from harvest year 2013 and 2014

Harvest year	Genotype		Crude protein content (% DM)	Sedimentation value (ml)	GMP (mg/g)	HMW (mg/g)	LMW (mg/g)	LMW/HMW ratio
2013	Julius	r	-0.27	-0.40	-0.40	-0.29	-0.43	-0.45
		p	n.s[(a)]	n.s	n.s	n.s	n.s	n.s
	Kompass	r	0.81	0.52	-0.29	-0.05	-0.37	-0.65
		p	0.027	n.s	n.s	n.s	n.s	n.s
	Dichter	r	0,34	0.43	0.70	0.81	0.72	-0.35
		p	n.s	n.s	n.s	0.027	n.s	n.s
	Attraktion	r	0.84	0.65	0.19	0.01	0.26	0.31
		p	0.009	n.s	n.s	n.s	n.s	n.s
	Diantha	r	-0.58	-0.54	0.05	0.00	0.03	-0.28
		p	n.s	n.s	n.s	n.s	n.s	n.s
	RGT Reform	r	0.85	0.74	-0.18	-0.39	-0.07	0.70
		p	0.008	0.038	n.s	n.s	n.s	n.s
	KWS Magic	r	0.66	0.54	0.01	-0.16	-0.04	0.25
		p	n.s	n.s	n.s	n.s	n.s	n.s
	KWS Loft	r	0.69	0.70	0.53	0.66	0.37	-0.51
		p	n.s	n.s	n.s	n.s	n.s	n.s
	KWS Montana	r	0.89	0.65	0.54	0.57	0.49	-0.38
		p	0.003	n.s	n.s	n.s	n.s	n.s
	Spontan	r	0.28	0.32	-0.19	-0.12	-0.14	0.02
		p	n.s	n.s	n.s	n.s	n.s	n.s
	Axioma	r	0.88	0.59	0.61	0.38	0.65	0.03
		p	0.004	n.s	n.s	n.s	n.s	n.s
	Jonny	r	0.05	0.32	-0.40	-0.39	-0.47	-0.06
		p	n.s	n.s	n.s	n.s	n.s	n.s
	Franz	r	0.86	0.46	0.47	0.59	0.45	-0.69
		p	0.006	n.s	n.s	n.s	n.s	n.s
	Bernstein	r	0.89	0.82	-0.10	0.09	-0.14	-0.36
		p	0.003	0.012	n.s	n.s	n.s	n.s
	Kobold	r	0.54	0.14	-0.32	-0.38	-0.29	0.25
		p	n.s	n.s	n.s	n.s	n.s	n.s
2014	Julius	r	0.72	0.66	0.25	0.16	0.27	0.27
		p	0.046	n.s	n.s	n.s	n.s	n.s
	Produzent	r	0.65	0.56	0.16	0.13	0.16	0.17

		p	n.s	n.s	n.s	n.s	n.s	n.s
	KWS Salix	r	0.92	0.85	-0.19	-0.19	-0.19	0.54
		p	0.001	0.007	n.s	n.s	n.s	n.s
	Bonanza	r	0.76	0.56	0.08	-0.12	0.13	0.87
		p	0.030	n.s	n.s	n.s	n.s	0.005
	Gustav	r	0.65	0.28	-0.32	-0.48	-0.28	0.65
		p	n.s	n.s	n.s	n.s	n.s	n.s
	Benchmark	r	0.84	0.73	0.17	0.21	0.15	-0.13
		p	0.010	0.039	n.s	n.s	n.s	n.s
	Faustus	r	0.54	0.46	0.07	-0.08	0.11	0.58
		p	n.s	n.s	n.s	n.s	n.s	n.s
	Ponticus	r	0.91	0.63	-0.21	-0.29	-0.18	0.27
		p	0.002	n.s	n.s	n.s	n.s	n.s
	Helmond	r	0.97	0.67	0.03	0.00	0.04	0.04
		p	<0.001	n.s	n.s	n.s	n.s	n.s
	Alexander	r	0.85	0.89	-0.04	-0.13	-0.02	0.24
		p	0.007	0.004	n.s	n.s	n.s	n.s
	Partner	r	0.64	0.51	0.25	0.22	0.25	0.33
		p	n.s	n.s	n.s	n.s	n.s	n.s

(a) $p > 0.05$, not significant (n.s)

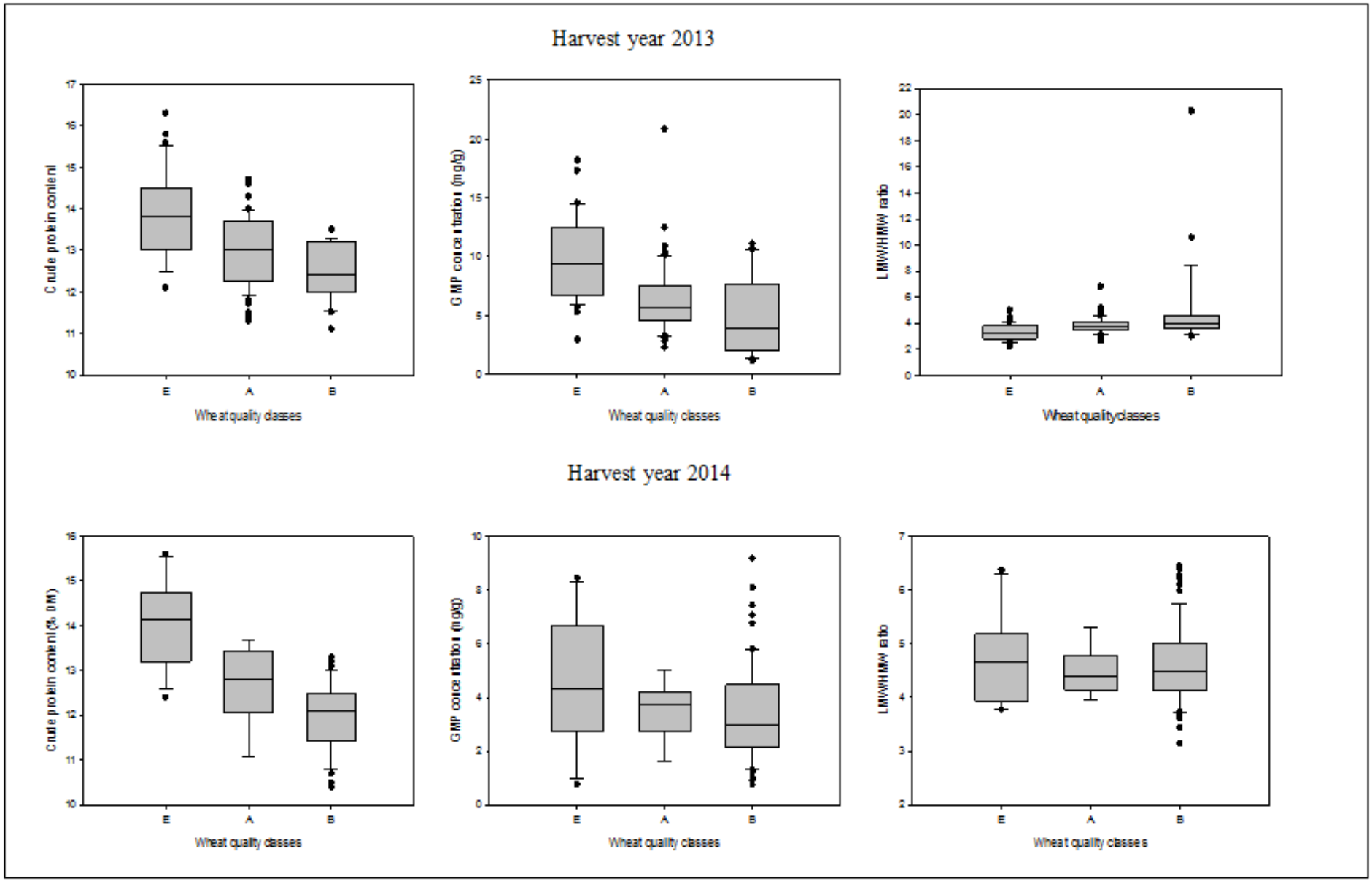


Figure 2: Crude protein content, GMP concentration and LMW/HMW ratio for wheat flour samples belonging to different quality classes (E, A, B) and harvest years 2013 and 2014.

4. Conclusions

This study demonstrates the impact of location and genotype on the GMP fractions and their ability to predict the loaf volume of wheat flour samples. GMP fractions concentrations of wheat flour samples from the harvest year 2013 were influenced more by genetic than environmental factors, in contrast with GMP fractions concentrations of wheat flour samples from the harvest year 2014, emphasising the major effect of environment and genotype on these fractions. The relationship between Loaf volume and GMP fractions concentrations of wheat flour samples from 2013 comes out in some cases significant and in others non-significant because it remains under the influence of the genetic background of the samples and the location of growth. This relationship is revealed non-significant for wheat flour samples from 2014, due to the heat effect, characterising this year, and which can lead to a decrease in GMP. This indicates that the GMP fractions concentrations cannot be a useful approach to replace the baking test and predict the loaf volume without considering the effect of the environment and genotype. An investigation of the composition of the GMP fractions might be also important to highlight the process involved in this relationship with loaf volume.

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III. Discussion and conclusions

The objective of the present article was to study the relationship between GMP, GMP subunits (HMW and LMW) contents of soft wheat varieties and their loaf volumes, while estimating the dependency of these gluten fractions on genotype and environemental conditions. Studies looking at this relationship based on a multigenotype and multilocation analysis, in order to integrate influences of location and genotype are not yet available. For this purpose, many different soft wheat flour samples from different locations in Germany (eight locations) and from two harvest years 2013 and 2014 were considered.

The outcome of this study demonstrated that for soft wheat flour samples of the harvest year 2013, genotype turned out to be a significant source of variation for all parameters examined; protein content, sedimentation value, loaf volume, GMP, HMW, LMW concentrations and LMW/HMW ratio, while the distinction between locations is possible only on the basis of protein content, sedimentation value and the ratio LMW/HMW. Moreover, location represented the largest source of variation for protein content ($H = 50.8$, $p \leq 0.001$) in comparison with quality class and genotype ($H = 25.7$, $p \leq 0.001$ and $H = 43.9$, $p \leq 0.001$, respectively), when genotype affected more sedimentation value, loaf volume, GMP, HMW, LMW concentrations and LMW/HMW ratio. This outcome is confirmed by the studies of Johansson et al. (2001), and Zhu and Khan (2001) who reported that protein concentration is determined both genetically and environmentally and that gluten macro polymer was controlled more by genetic than environmental factors, respectively.

However, between the different genotypes and quality classes of soft wheat flour samples deriving from the harvest year 2014, we didn't obtain a significant difference for GMP, HMW, LMW concentrations and LMW/HMW ratio, which could be explained by the heat effect on GMP and GMP subunit, which is attributed to the environmental conditions characterising the harvest year 2014 in Germany (Deutscher Wetterdienst, 2014). However, a significant difference was demonstrated among locations for GMP, GMP subunits concentrations and protein content, indicating that the location of growth affected these parameters. This difference seen between wheat samples from 2013 and 2014 may be attributed to the high temperatures, affecting wheat grain in terms of various physiological and biochemical processes, and which depends on the responsiveness and sensitivity of each genotype to the environment (Iqbal et al., 2017; de Leonardis et al., 2015). This heat effect was also highlighted, when flour samples from the harvest year 2013 showed higher GMP

concentrations in comparison with flour samples from 2014. As reported by Cairns et al., 2012, increased temperature during grain filling induces disturbances by the GMP particle

As reported by previous studies (DuPont and Altenbach, 2003; Cairns et al., 2012) heat stress induces disturbances by the GMP particles formation.

As reference to the relationship between GMP, GMP subunits concentrations, LMW/HMW ratio and loaf volume, results from this study demonstrated that GMP, HMW and LMW concentrations, except LMW/HMW ratio of wheat flour samples from 2013 showed positive correlation with loaf volume, while wheat samples from 2014 didn't show any significant relation to loaf volume. The highest coefficient of correlation obtained in 2013 was achieved with HMW concentration ($r = 0.55$, $p < 0.001$). This outcome isn't in accordance with the results obtained by Thanseauser et al. (2014) who obtained a close relationship between GMP, and GMP subunits concentrations and loaf volume (GMP: $r = 0.80$, HMW: $r = 0.77$ and LMW: $r = 0.77$). This difference might be due to the impact of kneading applied during the different baking tests used in both studies on GMP network, as well as du to the different the differences in plant material preparation.

However, this relationship between GMP fractions and loaf volume does not reach the correlation values obtained between sedimentation value, protein content and loaf volume. Moreover, it remains varying according to the different quality classes, locations and genotypes.

Soft wheat flour samples from 2013 revealed a positive significant correlation between GMP, HMW, LMW concentrations over all locations (except location 2), and a negative correlation between loaf volume and LMW/HMW ratio. These results are confirming the outcome of Jin et al. (2011), Dhaka and Khatkar (2014) who suggested that HMW plays a positive role in dough rheological properties by contributing more to dough strength than the LMW, Moreover, among locations and genotypes the highest correlation achieved with loaf volume fluctuates from GMP, HMW, LMW to LMW/HMW ratio. Those findings indicate that the relationship between loaf volume and GMP subunits concentrations significantly changed with location and genotype, confirming previous study, which supposed that environment and genotype affected the formation of GMP by modifying molecular sizes of glutenin polymers by influencing the rate of protein synthesis and incorporation of HMW and LMW subunits. (Zhu and Khan, 2001). In contrast almost, no location and genotype in 2014 represents a significant correlation between loaf volume and GMP subunits, which might be explained by the effect of heat on the genotypes present in these locations.

Chapter 5

Prediction of blending effect and baking quality of soft wheat blends by means of Extensograph, GlutoPeak®-Test and glutenin macro polymer under variable fertilisation levels and environmental conditions

I. Introduction

In different countries, wheat flours used are so far wheat blends obtained by blending wheat varieties with different quality and characteristics. Mixing of cultivars that complement each other has potential benefits, among others; it enables the increase of crop productivity, better pest and diseases resistance and permit to meet the specific production requirements, in consideration of baking quality (Gallandt et al., 2001; Cox et al., 2004). By these wheat blends, a noticeable synergistic effect is observed, known as blending effect. This effect resulted in a higher or lower baking quality as expected, that means in comparison with to the loaf volume mathematically assumed from the loaf volumes of the wheat flours blended. A blending effect is considered significant if a flour blend displays a loaf volume exceeding the theoretical volume by at least 15 ml / 100 g of flour using the Rapid-Mix-Test (Boiling, 1968; Arbeitsgemeinschaft Getreideforschung, 2016; Pelshenke et al.,2007).

A few of the available studies enquired into the clarification of the blending effect, its causes and its influences on the dough properties and on the baking performances. Some available studies supposed that this effect could be a result of synergistic effects of the different protein qualities of the wheat flours blended and doesn't depend on their protein content (Bushuk et al., 1983). While others reported that the blending effect and the rheological dough properties are closely related to each other, and that a complementarity between wheat varieties is achieved (Bolling, 1980; 1983; Schäfer and Flechsig, 1985). That means the determination of the relationship between the rheological dough properties through empirical instruments, the protein quality and the blending effect seems to be of major importance in order to highlight the influence of the blending effect on the baking performances.

This investigation should be carried out while taking into consideration the different parameters that can influence the blending effect, the protein quality, the dough rheology and consequently the baking quality, and which are the location of growth, the genotypic background and the nitrogen fertilisation.

The use of nitrogen fertilisers is nowadays considered as a necessity, because of the increasing requirement for specific wheat varieties possessing high protein content. This is because that baking performances of soft wheat cultivars are since many years known to be mainly determined by the quantity of grain proteins, and the higher prices are offered to farmers for wheat varieties that have, amongst other things, protein contents above a certain level.

Normally wheat nitrogen fertilisation is split into three applications at the beginning of the vegetation wheat growth stages to ensure an adequate grain yield, followed usually by late-season nitrogen fertilisation to increase grain protein content (Olson and Kurtz, 1982). The reason why, the timing and the rate of nitrogen fertilisation are recognized as being crucial factors in growing wheat varieties for high protein content. Nitrogen represents a primary component of protein; subsequently levels of soil available nitrogen have a large influence on yield, grain protein content, and subsequently on baking performances (Eilrich and Hageman, 1973).

However, it has been found that that in some cases baking quality didn't because increase in proportion to crude protein content, which was clearly revealed particularly when some modern approved wheat varieties from Germany are considered. These wheat varieties have high functionality of the gluten proteins leading to much better baking performance than expected from classical quality parameters, although relatively low grain proteins contents (Lindhauer, 2014). In addition to that, the late season nitrogen fertilisation may cause potential ecological problems, and its practical problems and extra costs (fertilizers, manpower, energy...) are undisputed. Therefore, it is primordial to investigate the effect of late nitrogen fertilisation on bread making performances more comprehensively, in order to be reduced or removed without decreasing wheat quality (Foulkes et al., 2009).

Accordingly, the present study has been the subject of the determination of the effect of location, genotype and late fertilisation level on the blending effect of soft wheat flour blends and on its relationship with the aggregation gluten properties, the rheological dough properties and the GMP content. Afterwards take advantage of it for the purpose of predicting loaf volume of wheat flour blends, which will be of economic benefit to farmers and help to reduce environmental effects associated with nitrogen fertilisation. Therefore, a set of six soft wheat varieties, harvested at three locations in Germany from Lower Saxony and Saxony-Anhalt and with three different late-season nitrogen fertilisation levels, low (160 Kg N/ ha), medium (180 Kg N/ ha) and high (200 Kg N/ ha) were blended with 50% to 50% level of mixture. The pure and the blend soft wheat flour samples were tested by means of GPT measuring the aggregation behaviour of wheat gluten, Extensograph assessing the dough rheological properties and their GMP contents were extracted under reducing conditions and quantitated by means of HPLC.

II. Article 3

Prediction of blending effect and baking quality of soft wheat blends by means of Extensograph, GlutoPeak®-Test and glutenin macro polymer under variable fertilisation levels and environmental conditions.

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Abstract

The blending effect as a synergistic effect obtained by wheat flour blends was investigated on the basis of a multigenotype, multilocation analysis and different nitrogen fertilisation levels (low, medium and high). Relationships between protein content, sedimentation value, GPT parameters, GMP subunits concentrations, Extensograph parameters and blending effect value (BEV) were evaluated, as well as loaf volume of wheat blends. Genotype, location, fertilisation level and the interaction location × fertilisation level affected significantly the BEV, but the transition from medium to high fertilisation level over all locations didn't have any effect. The difference between the HMW values of the blending partners (ΔHMW) presented the highest correlation with BEV ($r = 0.65$, $p < 0.001$ at high level), but fertilisation level has an influence on the stability of this relationship. Among the Extensograph parameters, the mean value of the resistance to extension of the blending partners at 45 min ($R_{\bar{X}}$ (45min)) showed the highest significant and environmental stable correlation with blends loaf volume ($r = 0.67$, $p < 0.001$ at low level; $r = 0.75$, $p < 0.001$at medium level and $r = 0.74$, $p < 0.001$ at high level). A linear function of $R_{\bar{X}}$ (45 min), the mean value of the GMP concentrations of the blending partners ($GMP_{\bar{X}}$), protein content and sedimentation value can

provide an explanation of loaf volume variation by 69% with an uncertainty of ± 23 ml. The evaluation of this model resulted in good correlation between observed and predicted loaf volume over the three fertilisation levels ($r = 0.89$, $p < 0.001$ at low level; $r = 0.84$, $p < 0.001$ at medium level and $r = 0.83$, $p < 0.001$ at high level), which emphasises that this model could be useful to explain variation in loaf volume of blends whichever is the level of fertilisation, and subsequently predict the BEV that is normally noticed only by means of the baking tests.

Keywords: Blending effect, loaf volume, location, genotype, nitrogen fertilisation level.

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops and covers worldwide more cultivated land than any other crop. It is ranked second in the total cereal production behind coarse grains and before rice (FAO, 2016) and consumed as primary food for the majority of the world's inhabitants (Waines and Hegde, 2003). Because of its fundamental role in the global food security and the world economy, the forecasts demand for 2020 of wheat to fulfil the need of growing world population varies between 840 and 1050 million tons of wheat (Russell et al., 2000).

Due to the presence of gluten proteins in wheat flour, main source of the viscoelastic properties of wheat flour dough, the greatest proportion of wheat flour produced is used for bread making. Many efforts have been made and a great attention was paid over years to study the baking quality of wheat and to predict it. Grain protein content, as an indirect parameter for baking quality, is globally considered the main criterion in assessing wheat baking quality (Wrigley and Bietz, 1988), which leads to an increasing demand for specific wheat varieties characterised by high grain protein content. For that reason, nitrogen fertilization is practiced as a common strategy that can influence the storage proteins and the rheological properties of wheat flour (Flagella, 2006; Fuertes-Mendizábal et al., 2010). Nitrogen is a primary component of protein, and therefore, levels of soil available nitrogen have a large influence on grain protein content (Eilrich and Hageman, 1973). However, nitrogen fertilisation represents a significant cost of production for the farmers and has negative environmental impacts through nitrate leaching, and N_2O emissions associated with denitrification (Foulkes et al., 2009). Furthermore, it has been already demonstrated that crude protein content shows low correlation with the baking quality, particularly when some modern approved wheat varieties from Germany are considered. These wheat varieties have high functionality of the gluten proteins leading to much better baking performance than expected

from classical quality parameters, although relatively low grain proteins contents (Lindhauer, 2014). Therefore, Seling (2010) and Kazman (2010) provide evidence for the assumption that the classical quality parameters appear to be no longer suitable to assess the baking quality of modern German wheat varieties. In addition to that, generally wheat flours used are so far wheat blends obtained by blending wheat varieties with different quality and characteristics, in order to improve and reach the requested baking quality, and subsequently meet the customer's specifications.

By wheat blends composed of two wheat varieties or more, a noticeable synergistic effect is observed. This effect resulted in a higher or lower baking quality as expected, which means the obtained loaf volume, can exceed or be inferior to the loaf volume mathematically assumed from the loaf volumes of the wheat flours blended. This effect is known as blending effect. According to Bolling (1968), a blending effect is considered if a flour blend displays a loaf volume exceeding the theoretical volume by at least 15 ml / 100 g of flour using the Rapid-Mix-Test (Arbeitsgemeinschaft Getreideforschung, 2016; Pelshenke et al.,2007) as a baking test.

Nevertheless, a few of the available studies enquired into the clarification of the causes of the blending effect and its influences on the dough properties and the baking performances. Bushuk et al. (1983) supposed that this effect could be a result of synergistic effects of the different protein qualities of the wheat flours blended and doesn't depend on their protein content. While Bolling (1980; 1983) reported that the blending effect and the rheological dough properties are closely related to each other, indicating that complementarity between wheat varieties is achieved. Thus, Schäfer and Flechsig (1985) demonstrated that wheat varieties with soft dough properties are strengthened by the means of wheat varieties with strong dough properties, dealing to an increase of the loaf volume of the wheat blends, and the more different the stretch resistance and elasticity dough properties of the wheat flours blended is the greater blending effect is obtained.

In addition to that, it is well known that characterizing the rheological properties of dough through empirical instruments is effective in predicting its processing behaviour and obtaining information on the baking quality of the respective flour, by simulating the bread making steps (Autio et al., 2001; Chiotelli et al., 2004; Collar et al., 2007). Among these equipments, Extensograph, Farinograph and Mixograph are the most common used to characterise dough rheology, as well as Alveograph in some countries (Song and Zheng, 2007).

Besides, gluten fractions, glutenins and gliadins, have been reported to have a prominent role in the dough strength and the viscous dough properties, respectively (MacRitchie, 1980). A crucial fraction of the glutenins can be isolated from wheat flour as a SDS insoluble gel layer, called gluten macro polymer (GMP) (Graveland et al., 1982) has been shown as an important factor in assessing and predicting rheological wheat dough properties (Kelfkens and Lichtendonk, 2000; Don et al., 2003b), and which influences dough strength, resistance to extension and dough development time (Moonen et al., 1982; MacRitchie and Lafiandra, 1997; Don et al., 2003a). Dough extensibility and tenacity have been also investigated and predicted by means of the GlutoPeak®-Test (GPT) (Marti et al., 2015), which is an equipment recently proposed for the evaluation of flour quality, measuring the aggregation behaviour of wheat gluten.

Therefore, the objective of this study was to investigate the relationship between blending effect, the aggregation gluten properties through GPT, the rheological dough properties by means of the Extensograph and the GMP content of wheat flour blends, in order to assess the blending effect and afterwards take advantage of it for the purpose of predicting loaf volume of wheat flour blends, which will be of economic benefit to farmers and help to reduce environmental effects associated with nitrogen fertilisation. For this purpose, a set of six soft wheat varieties, harvested at three locations in Germany and with three levels of nitrogen fertilisation were blended with 50% to 50% level of mixture. The pure and the blend soft wheat flour samples were tested by means of GPT, Extensograph and their GMP content.

2. Material and methods

2.1. Plant material

Six different soft wheat varieties from the harvest year 2015 cultivated at three different locations in Germany (Lower Saxony and Saxony-Anhalt) and with three different levels of nitrogen fertilisation (low, medium and high level of fertilisation) were investigated in this study. All varieties received the same amount of nitrogen fertilizer at the beginning of the vegetation (50 Kg N/ ha), but different late-season nitrogen fertilisation levels, low (160 Kg N/ ha), medium (180 Kg N/ ha) and high (200 Kg N/ ha).

The wheat varieties are classified according to the German Descriptive Variety List in three groups: Elite wheat (E-class) characterised by the highest quality: n = 1 (Kerubino), followed by Quality wheat (A-class): n = 3 (JB Asano, Julius and Opal), and Bread wheat (B-class): n = 2 (Rumor and Memory), C-class (not useable for baking) was not included in this study. The milling of wheat grains samples into flour was performed using a Buehler laboratory mill

MLU 202 and the flour samples produced were flour type 550, according to DIN 10355 Standard with approximately 0.60 % ash content, determined according to ICC Standards Methods (ICC 104/1), as recommended by Arbeitsgemeinschaft Getreideforschung, (2016).

All wheat varieties from the three locations and the three levels of fertilisation were blended to each other, within location and level of fertilization after milling into flour to a ratio of 50:50. This study included 54 pure wheat flour samples and 135 blend wheat flour samples.

2.2. Standard methods

Protein content was determined using Near Infrared Transmission spectroscopy (NITs) with Infratec™ 1241 (Foss GMbH, Rellingen, Germany), according to DIN EN 15948:2012 (protein content % expressed on a dry matter basis (% DM)). Zeleny Sedimentation value was determined according to ICC 116/1, after preparing the flour samples according to ICC 118. Protein content and sedimentation value of wheat flour blends were mathematically calculated according to the values of the pure flour samples.

2.3. Rapid Mix Test

Rapid mix Test (RMT), as a standard baking test, was performed according to the method of (Arbeitsgemeinschaft Getreideforschung, 2016; Pelshenke et al.,2007) to produce bread rolls in order to measure the loaf volume of pure flour samples and blends flour samples. Before the dough preparation, the moisture content was determined with the NIT Spectroscopy of the whole grain with Infratec™ 1241(Foss GMbH, Rellingen, Germany) according to the DIN EN 15948:2012, the falling number according to Hagberg- Perten DIN EN ISO 3093:2009, and finally the water absorption (ml/100 g) determined using the Farinograph (Brabender GmbH and Co KG, Duisburg, Germany) according to ICC 115/1.

2.4. GlutoPeak®-Test

The assessment of the gluten aggregation properties of pure flour samples and blends flour samples was carried out using the GlutoPeak®-Test (Brabender GmbH and Co KG, Duisburg, Germany). 10.2 g flour and 11.2 g 1.0 % sodium chloride solution (w/w) were added into the sample cup at 36 °C, and with a paddle speed of 2750 rpm.
All measurements were performed for 300sec in duplicates.
The parameters measured automatically by the GPT software are: Maximum Torque (MT) expressed in Brabender Units (BU) corresponding to the peak occurring as gluten aggregates,

Peak Maximum Time (PMT) expressed in seconds corresponding to the time at peak torque, AM and PM expressed in BU corresponding to the torque 15 seconds before the PMT and to the torque 15 seconds after the MT, respectively. The five areas automatically calculated by the software and corresponding to the energy required for gluten aggregation (A0-A1, A1-A2, A2-A3, A3-A4 and A4-A5) were not taken into consideration in this study, because as reported by Bouachra et al. (2017) these GPT areas using the previous described method didn't display a significant relation with the baking quality, in contrast with the parameter AM, which highlighted the highest correlation with loaf volume.

2.5. Extraction and quantitation of GMP

GMP of flours (pure and blend flour samples) were extracted and quantitated by means of HPLC following the procedure described by Thanhaeuser et al. (2014).

Different chemicals were required for the extraction of GMP: SDS Pellets ≥ 99,5% Rotiphorese-Grade, Hydrochloric acid fuming (HCl) ROTIPURAN® 37%, p.a., and 1,4-Dithiothreitol ≥ 99% (DTT), p.a., were from Carl Roth GmbH + Co KG (Karlsruhe, Germany), di-Sodium hydrogen phosphate dehydrate (NaH2PO4), Potassium dihydrogen phosphate (HKNaPO4) and 1-Propanol for analysis EMSURE® ACS,Reag. Ph Eur. ≤ 100% were purchased from Merck KGaA (Darmstadt, Germany).

100 mg of flour was weighted into a Pyrex tube (capacity: 12 mL), then extracted twice at room temperature with solvent A (1% (w/v) SDS and 0.05 mol/L NaH2PO4, (pH 6.9); the pH value was adjusted with HCl (32%, w/w)), and twice at 60° C with solvent B (50% (v/v) 1-propanol, 0.05 mol/L HKNaPO4 (pH 7.5) and 1% (w/v) DTT) under nitrogen. Each extraction step was initiated with vortexing for 2 min at room temperature and followed by magnetic stirring for 30 min (at room temperature when solvent A was used, and at 60° C when solvent B was used) and continued by a centrifugation of the suspension for 25 min at 4600 rpm/min at room temperature (Heraeus Megafuge 1.0, Thermo Fisher Scientific, Dreieich, Germany). The supernatants were transferred to a volumetric flask (capacity: 2 mL) and diluted to 2 mL with solvent B. The extracts were filtered through a 0.45µm filter, transferred into the HPLC vials.

A Kontron HPLC instrument (Kontron Instrument, Roßdorf, Germany) fitted with a Yarra 3u SEC-3000 column (Phenomenex, Aschaffenburg, Germany) was used to analyse the extracts under the following conditions: The column temperature was the room temperature, the injection volume was 20 µL, the eluent was solvent A at a flow rate of 0.3 mL/min, and detection was made by measuring the UV absorbance at 210 nm by means of a Kontron 332

UV-Vis-Detector. The integration of the obtained areas was performed using Chromgate software (Knauer, Berlin, Germany).
As a reference material for the calibration, gliadin reference from the Prolamin Working Group (PWG gliadin) (van Eckert et al., 2006) was used.
The extractions and measurements were carried out for each flour sample in duplicate.

2.6. Extensograph

The dough rheological properties of the pure flour samples and blends flour samples were tested by means of the Extensograph (Brabender GmbH and Co KG, Duisburg, Germany) according to ICC 114/1 with some modifications during the dough preparation, in order to get the physical dough characteristics obtained in the RMT. Thus, in addition to distilled water and 2 % Sodium chloride, 0.002 % Ascorbic acid was added to 300g wheat flour (14 % moisture content) in the determination of the necessary amount of water to achieve a dough consistency of 500 BU after 5 min mixing using the Farinograph (Brabender GmbH and Co KG, Duisburg, Germany) and according to ICC 115/1. After mixing and modelling, samples are measured 3 times, each after a 45 min proving time at 30 °C and 100 % RH that means after 45, 90 and 135 min. All measurements were performed in duplicates and the following determinations were made:
Energy (En) expressed in $cm^{2,}$ Resistance to extension (R) expressed in Brabender Units (BU), Extensiblity (E) expressed in mm, and R/E Ratio.

2.7. Statistical analysis

Statistical analysis was performed using SigmaPlot for windows version 14 (Systat Software Inc., San Jose, USA).
Two-way ANOVA was carried out to determine significant differences in the measured parameters according to location of growth, genotype, fertilisation level and their interactions. A multiple comparison statistical procedure using Holm-Sidak Test as Post Hoc Test was used to highlight where the differences occurred between groups at a significance level of $\alpha = 0.05$.
A linear Pearson correlation was carried out to assess the relationship between the measured parameters based on the means of all blends and means of all genotypes and locations for each level of fertilisation. Finally, to develop an effective predictive model for the dependent variables, best subset regression analysis was applied.

3. Results and Discussion

3.1.Composition of the wheat flour samples and their blends

The ranges values of the physico-chemical parameters measured of the tested pure and blend flour samples from three locations and three levels of fertilisation are shown in Table 1. The crude protein content and the sedimentation value of pure and blend flour samples from locations 1 and 3 increase with increasing fertilisation level, which is confirmed by the studies of Gauer et al. (1992) and Zecevic et al. (2010). For example, in location 1 crude protein content ranges from 12.1 to 13.7 % DM applying the lowest level of fertilisation, from 12.4 to 13.8 % DM with medium level and from 13.0 to 14.5 % DM with high level.

Those flour samples reacted otherwise to the different levels of fertilisation in location 2; the highest values of crude protein content and sedimentation value were obtained with the low nitrogen fertilisation level, followed by the high level and finally the medium level.

Differently from the interrelation observed between the nitrogen fertilisation levels and loaf volume of blend flour samples in location 1 (524 - 610 ml/100 g (low), 530 - 639 ml/100 g (medium) and 580 - 670 ml/100 g (high)), loaf volume of pure and blend flour samples in locations 2 and 3 didn't vary according to the levels of fertilisation. These results are in contrast with the finding of Garrido-Lestache et al. (2004) and Fuertes-Mendizábal et al. (2010), who demonstrated that wheat bread making quality was improved with higher nitrogen fertilisation level. However, the studies of Xue et al. (2016) and Brabant and Häner, (2016) demonstrated that the application of the late season nitrogen fertilisation doesn't improve the baking quality, which confirm our results. Furthermore, it was also highlighted that the effect of nitrogen fertilisation varied between different environmental conditions such as locations (Jia et al., 1996), which can explain the results obtained for loaf volume of pure and blend flour samples in locations 2 and 3, and of pure flour samples in location 1.

Table 1: Ranges of protein content, sedimentation value, loaf volume, GPT parameters, GMP subunits concentrations and Extensograph parameters at three proving times (45 min, 90 min, 135 min) of pure flour samples (n = 54) and blend flour samples (n = 135) harvested at three locations in Germany and at three fertilisation levels (low, medium and high).

	Location		Location 1			Location 2			Location 3		
Samples	Level of fertilisation		Low	Medium	High	Low	Medium	High	Low	Medium	High
Pure flour samples n = 54	Quality Parameters:										
	PC [a] (% DM)		12.1 - 13.7	12.4 - 13.8	13.0 - 14.5	13.1 - 15.2	12.1 - 13.8	12.8 - 14.9	11.7 - 13.6	12.5 - 14.6	13.0 - 14.7
	SV [b] (ml)		37 - 63	40 - 69	41 - 72	44 - 71	37 - 54	43 - 66	34 - 60	37 - 70	39 - 73
	LV [c] (ml/100g)		532 - 633	498 - 652	537 - 641	545 - 670	579 - 670	590 - 662	485 - 637	494 - 627	489 - 659
	PMT (s)		52 - 89	44 - 92	46 - 68	34 - 70	41 - 81	35 - 78	53 - 89	35 - 90	47 - 93
	MT (BU)		54 - 70	55 - 73	60 - 75	60 - 82	51 - 75	55 - 80	46 - 72	51 - 79	53 - 72
	AM (BU)		21 - 30	22 - 31	25 - 32	26 - 31	25 - 30	26 - 32	21 - 30	20 - 33	21 - 30
	PM (BU)		46 - 57	46 - 56	47 - 61	50 - 63	45 - 59	48 - 61	41 - 56	44 - 64	45 - 58
	GMP (mg/g)		2.46 - 9.05	3.11 - 5.48	3.46 - 9.24	5.11 - 9.57	2.93 - 6.65	3.30 - 6.69	2.84 - 5.80	2.77 - 4.77	3.08 - 4.43
	HMW (mg/g)		0.46 - 1.55	0.36 - 0.88	0.58 - 1.42	0.69 - 1.33	0.27 - 0.92	0.32 - 0.56	0.43 - 0.74	0.33 - 0.45	0.44 - 0.47
	LMW (mg/g)		2.16 - 7.66	2.74 - 4.76	3.04 - 7.98	4.52 - 8.40	2.74 - 5.88	3.12 - 6.28	2.57 - 5.22	2.48 - 4.51	2.80 - 4.14
	LMW/HMW		4.73 - 6.01	5.23 - 12.97	5.23 - 6.24	6.09 - 7.01	6.38 - 11.40	9.24 - 11.35	5.97 - 8.43	5.57 - 10.92	6.25 - 9.28
	En (cm2)	45 min	57 - 76	52 - 84	53 - 87	62 - 82	63 - 81	59 - 89	59 - 96	52 - 88	51 - 100
	R (BU)		178 - 282	160 - 278	156 - 280	202 - 312	211 - 316	194 - 306	176 - 289	162 - 272	146 - 282
	E (mm)		142 - 164	149 - 169	145 - 180	142 - 162	140 - 158	142 - 162	139 - 173	144 - 180	147 - 198
	R/E ratio		1.09 - 1.99	0.97 - 1.84	0.92 - 1.83	1.25 - 2.20	1.34 - 2.21	1.22 - 2.15	1.04 - 1.97	0.98 - 1.89	0.81 - 1.92
	En (cm2)	90 min	78 - 106	74 - 108	72 - 103	86 - 99	77 - 104	80 - 102	80 - 131	79 - 114	75 - 120
	R (BU)		287 - 580	247 - 496	238 - 518	306 - 508	340 - 530	274 - 522	309 - 540	250 - 502	214 - 513
	E (mm)		123 - 157	121 - 162	125 - 162	121 - 157	113 - 142	120 - 156	112 - 159	124 - 167	128 - 179
	R/E ratio		1.94 - 4.72	1.54 - 4.10	1.51 - 4.14	1.95 - 4.20	2.46 - 4.69	1.76 - 4.35	1.99 - 4.82	1.53 - 3.83	1.23 - 4.01
	En (cm2)	135 min	78 - 97	77 - 117	84 - 101	86 - 102	72 - 98	87 - 102	78 - 130	83 - 131	88 - 115
	R (BU)		368 - 652	289 - 621	298 - 618	342 - 594	406 - 580	323 - 586	409 - 608	307 - 546	262 - 608
	E (mm)		102 - 139	111 - 155	110 - 154	105 - 144	103 - 128	113 - 149	102 - 148	113 - 163	113 - 170
	R/E ratio		2.65 - 6.39	1.99 - 5.59	1.97 - 5.62	2.38 - 5.66	3.17 - 5.63	2.18 - 5.19	2.92 - 5.96	1.92 - 4.83	1.54 - 5.38
	PC [a] (% DM)		12.1 - 13.4	12.5 - 13.8	13.1 - 14.5	13.2 - 14.8	12.4 - 13.7	12.9 - 14.5	11.8 - 13.4	12.6 - 14.3	13.3 - 14.7
	SV [b] (ml)		38 - 61	40 - 64	43 - 67	46 - 69	37 - 54	43 - 65	35 - 56	38 - 65	47 - 69

Blend flour samples n = 135	LV [c] (ml/100g)		524 - 610	530 - 639	580 - 670	534 - 666	592 - 676	573 - 652	528 - 612	501 - 597	509 - 627
	PMT (s)		51 - 81	51 - 81	49 - 77	44 - 73	52 - 81	47 - 88	69 - 104	53 - 93	49 - 87
	MT (BU)		51 - 66	54 - 70	57 - 73	59 - 78	48 - 68	53 - 74	49 - 63	52 - 68	51 - 70
	AM (BU)		20 - 27	22 - 28	22 - 29	24 - 30	24 - 29	25 - 29	21 - 28	21 - 27	21 - 28
	PM (BU)		43 - 53	44 - 55	46 - 57	47 - 59	43 - 55	46 - 59	42 - 49	42 - 53	44 - 55
	GMP (mg/g)		3.13 - 7.11	2.82 - 6.91	3.35 - 8.03	4.89 - 8.72	3.95 - 7.49	5.12 - 8.44	3.77 - 7.76	3.78 - 8.22	2.93 - 7.87
	HMW (mg/g)		0.68 - 1.49	0.59 - 1.40	0.27 - 1.74	0.51 - 1.78	0.65 - 1.39	0.86 - 1.52	0.76 - 1.47	0.44 - 1.47	0.38 - 1.33
	LMW (mg/g)		2.60 - 5.81	2.39 - 5.70	3.23 - 6.45	4.30 - 7.24	3.46 - 6.27	4.41 - 7.08	3.17 - 6.44	3.50 - 6.92	2.71 - 6.70
	LMW/HMW		3.68 - 4.38	3.84 - 4.55	3.58 - 11.78	3.07 - 8.80	4.44 - 5.35	4.66 - 5.27	4.15 - 4.72	4.49 - 8.02	4.81 - 8.59
	En (cm2)	45 min	58 - 86	66 - 103	76 - 108	73 - 103	67 - 96	71 - 101	69 - 101	70 - 107	68 - 118
	R (BU)		249 - 344	246 - 386	235 - 382	255 - 361	272 - 381	270 - 381	285 - 402	244 - 369	250 - 390
	E (mm)		129 - 155	139 - 164	142 - 170	138 - 174	138 - 158	140 - 160	130 - 158	130 - 173	134 - 171
	R/E ratio		1.70 - 2.49	1.52 - 2.76	1.42 - 2.51	1.62 - 2.62	1.93 - 2.68	1.78 - 2.65	1.85 - 3.00	1.56 - 2.84	1.53 - 2.77
	En (cm2)	90 min	74 - 109	79 - 113	83 - 111	88 - 111	80 - 105	71 - 111	87 - 125	81 - 117	77 - 130
	R (BU)		353 - 526	300 - 518	302 - 511	352 - 558	386 - 526	288 - 511	384 - 582	344 - 516	308 - 566
	E (mm)		112 - 152	126 - 162	121 - 163	122 - 155	114 - 142	119 - 145	122 - 153	115 - 157	124 - 180
	R/E ratio		2.37 - 4.57	1.88 - 4.11	1.90 - 4.22	2.28 - 4.57	2.77 - 4.61	1.99 - 4.29	2.51 - 4.66	2.27 - 4.49	1.94 - 4.56
	En (cm2)	135 min	78 - 114	83 - 109	83 - 106	77 - 107	83 - 105	66 - 108	82 - 121	84 - 120	80 - 120
	R (BU)		387 - 551	320 - 551	332 - 558	356 - 592	412 - 593	294 - 544	416 - 622	360 - 548	326 - 598
	E (mm)		110 - 140	113 - 159	116 - 157	114 - 142	107 - 131	109 - 149	106 - 150	117 - 154	124 - 158
	R/E ratio		2.77 - 5.01	2.01 - 4.88	2.21 - 4.69	2.72 - 5.19	3.19 - 5.54	2.19 - 4.78	0.77 - 5.45	2.48 - 4.39	2.17 - 4.67

[a] Crude Protein content (% DM).

[b] Sedimentation value (ml).

[c] Loaf volume (ml/100g of flour)

3.2.Analysis of blending effect according to genotype, location and level of fertilisation

As reported by Bolling, 1968, different blending effects were obtained by wheat blends in this study. Three results were achieved and are illustrated in Fig. 1. In the three illustrations, the blending effect value (BEV) corresponds to the difference between the observed loaf volume of the blend obtained from the RMT baking test and the calculated loaf volume obtained from the loaf volume values of the pure flour samples integrated in this blend. For some blends, this BEV can be positive and indicated a positive blending effect, like in the case of JB Asano – Memory blend in location 1 at high fertilisation level (Fig. 1a). In another case, some blends highlighted a negative blending effect with a negative BEV, as demonstrated in Fig. 1b for JB Asano – Memory blend in location 1 at low fertilisation level. However, other blends didn't show any significant blending effect, which means the BEV turns out to be less than 15 ml / 100g of flour, for example JB Asano – Memory blend in location 1 at medium fertilisation level (Fig. 1c).

Fig. 2 exhibited the BEV of all blends from the three locations and at the three levels of fertilisation. Among the 135 blend flour samples, only 18 blends show a positive blending effect. These 18 blends are distributed over the 3 locations, in such a way that location 1 integrates 10 blends, 6 at high level of fertilisation and 4 at medium level. Moreover, location 1 presents the highest BEV (52 ml / 100g) with Kerubino – Memory blend obtained at medium fertilisation level. Location 2 contains 5 blends with positive blending effect and also displayed a high BEV (51 ml / 100g) with Memory – Julius blend at low fertilisation level, while location 3 has just 3 blend flour samples with positive blending effect. Those results indicated that location of growth represents a significant source of variation for the blending effect.

The results obtained according to genotypes indicated that among the 15 blend flour samples investigated in this study, 9 blends exhibited a positive blending effect (Fig. 2), while 6 blends didn't show any blending effect or showed a negative effect over the three locations and at the three fertilisation levels. All blends involving Memory as a blending partner had a positive BEV at least in one location and at one level of fertilisation (Kerubino – Memory, Opal – Memory, JB Asano – Memory, Memory – Rumor and Memory – Julius). The same case is noticed for blends involving Kerubino variety, except Kerubino – JB Asano blend. That can be due to the presence of JB Asano variety in this blend, which is involved only in blends showing either negative blending effect or no effect, with the exception of JB Asano – Memory blend.

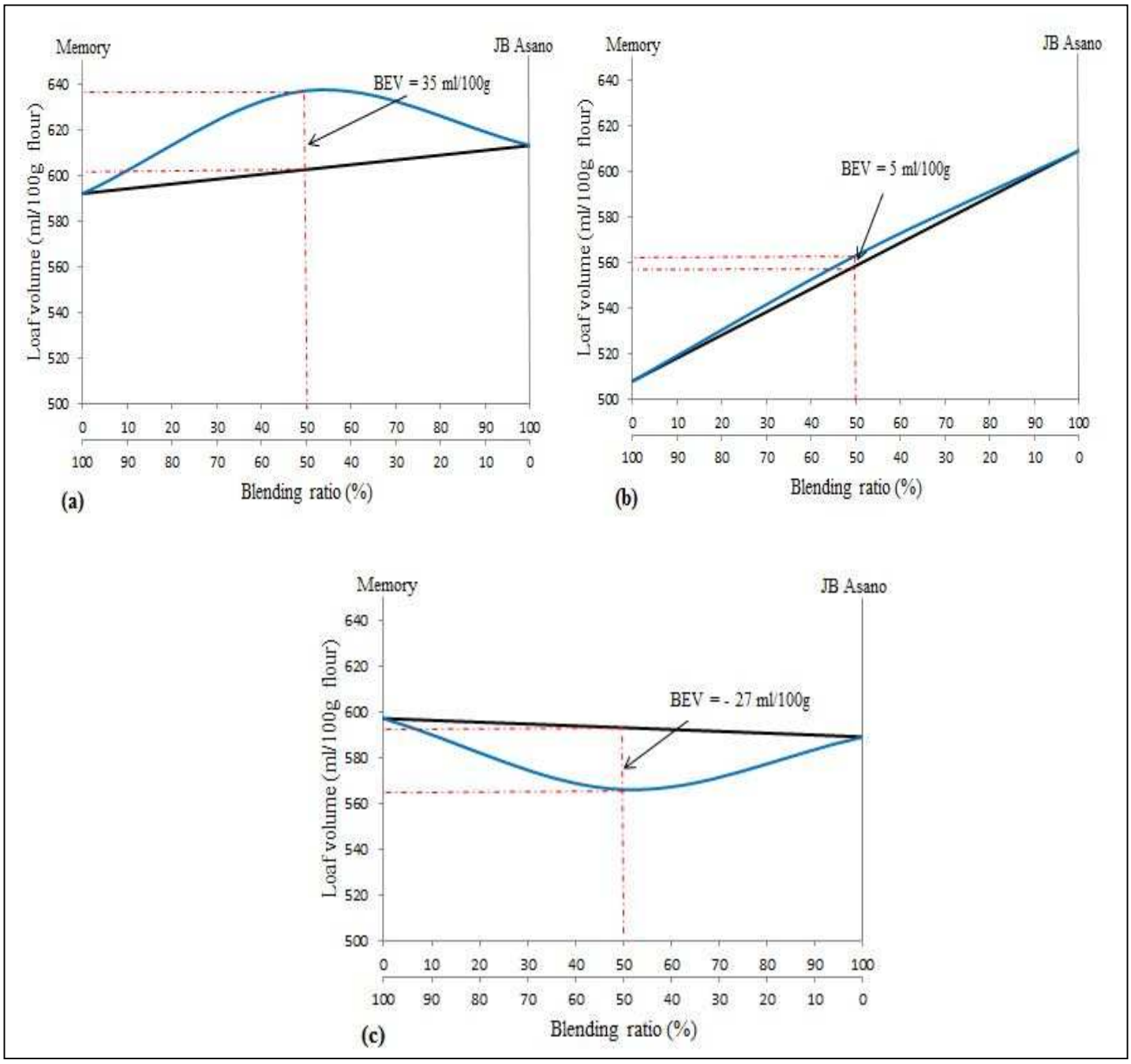


Figure 1: Example of the three types of blending effect obtained. (a) Positive BEV by Memory – JB Asano blend in location 1 at low fertilisation level, (b) BEV < 15 ml/100g by Memory – JB Asano blend in location 1 at medium fertilisation level, (c) Negative BEV by Memory – JB Asano blend in location 1 at high fertilisation level.

Regarding to the levels of fertilisation applied in this study, the 18 blend flour samples indicating a positive blending effect are distributed over the three locations in such a way that the number of blends is increasing with the increase of fertilisation level (4 blends at low level, 6 blends at medium level and 8 blends at high level). The blend Kerubino – Memory had the highest BEV (52 ml / 100 g) in location 1 at medium fertilisation level, however in location 2 the BEV at medium level (31 ml / 100 g) is twice the BEV at low level (15 ml / 100 g) as reported in Fig. 2 also. In location 1, the blend Memory – Rumor displayed a high BEV at medium level (47 ml / 100 g) and decreased at high level to reach 17 ml / 100 g. Those results highlighted the large influence of location and genotype and their interaction on the blending effect over the different fertilisation levels, which is needful to elucidate. However, it seems that the late nitrogen application (high fertilisation level) doesn't have an impact on the BEV, which achieves the highest values at medium fertilisation level. This observation appears to be confirmed by the stability of the BEV of the blend Memory – Julius over the medium and high fertilisation levels in location 1 (42 ml / 100 g and 41 ml / 100 g, respectively), nevertheless this stability might be also the results of the complementarity between the blending partners Memory and Julius, which are the varieties with the most complementary dough properties, as demonstrated by the Extensograph data (data not shown). Analysis of variance showed significant differences among genotypes, locations and fertilisation levels of blends on the BEV (Table 2). Among the blends studied Memory – Julius, Kerubino – Memory and Rumor – Julius differed significantly from other blends involving JB Asano or/and Opal as blending partner. These three blends highlighted positive high BEV at least over two locations. Differences were found among locations for BEV, except between location 1 and location 2. Concerning BEV over fertilisation levels, no significant difference was obtained between high and medium level, in contrast with the high – low and medium – low. This observation means that a high amount of nitrogen fertilisation doesn't apply automatically a positive and high BEV, because medium fertilisation level can lead to the same blending effect achieved with high level. However, this BEV appears to be also conditioned by the interaction location × fertilisation level ($p = 0.008$) as we can see in Table 2. That means the magnitude of the significant difference between the different fertilisation levels depends on which location, the blending partners composing the blends are from. Over the 3 locations, only in location 1 a significant difference was observed between high and low level and between medium and low level. Regarding medium vs. high, any significant difference was obtained over the 3 locations. These results are confirming our previous observation and approve that a reduced nitrogen fertilisation from high to medium

level doesn't impact the BEV and the same result can be achieved either with medium or high fertilisation level. The other interactions genotype × fertilisation level and genotype × location turned out to be not significant ($p = 0.728$; $p = 0.853$, respectively). Our outcome cannot be compared with other studies, because the effect of genotype, location and fertilisation level on blending effect has not been previously investigated.

Bolling, (1984a) noted that the blending effect can be clarified by the rheological dough properties, which depend on the genotype characteristics and location conditions influences.

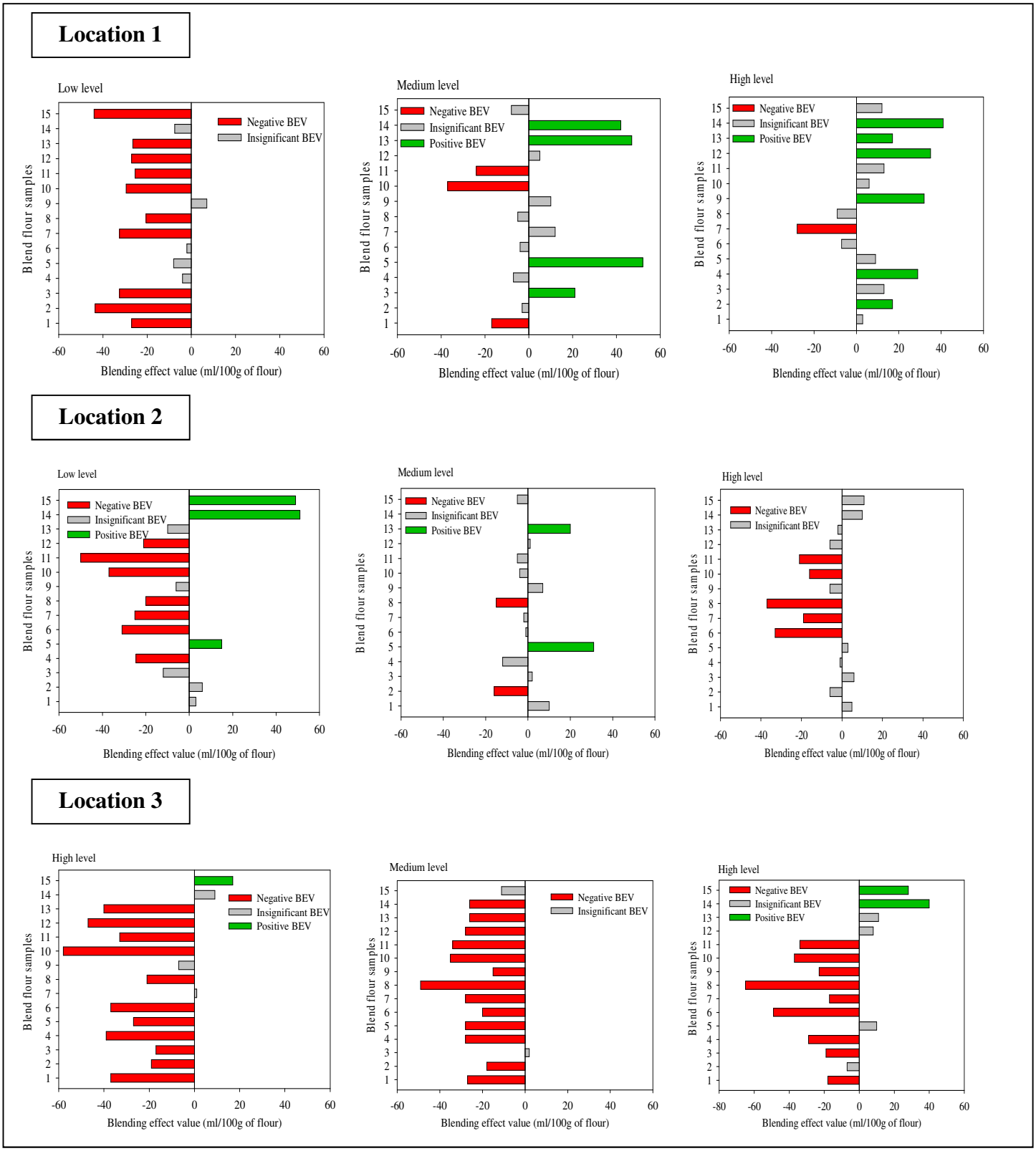


Figure 2: Blending effect values (BEV) of blend flour samples, from three locations and at three fertilisation levels (low, medium and high), (Negative BEV ≤ -15 ml/100; - 15 ml/100g < Insignificant BEV < 15 ml/100g; Positive BEV ≥ 15 ml/100g). Blend flour samples, 1 : Kerubino - JB Asano, 2 : Kerubino - Rumor, 3 : Kerubino - Julius, 4 : Kerubino - Opal, 5 : Kerubino - Memory, 6 : Opal - JB Asano, 7 : Opal - Rumor, 8 : Opal - Julius, 9 : Opal - Memory, 10 : JB Asano - Rumor, 11 : JB Asano - Julius, 12 : JB Asano - Memory, 13 : Memory - Rumor, 14 : Memory - Julius, 15 : Rumor – Julius.

Table 2: Analysis of variance of blending effect value and significant different pairs ($p < 0.05$) resulting from Holm-Sidak Test according to genotype, location, fertilisation level and their interactions.

Source	df [a]	F	p
Genotype	14	4,639	<0,001
Location	2	14,308	<0,001
Ferilisation	2	8,168	<0,001
Genotype x Location	28	0,704	0,853
Genotype x Fertilisation	28	0,813	0,728
Location x Ferilisation	4	3,605	0,008
	Holm- Sidak Test		
Groups	t	P	P<0,050
Genotype*			
Memory - Julius vs. JB Asano - Rumor	5,262	<0,001	Yes
Memory - Julius vs. Opal - Julius	5,185	<0,001	Yes
Memory - Julius vs. JB Asano - Julius	4,823	<0,001	Yes
Memory - Julius vs. Opal - JB Asano	4,435	0,002	Yes
Kerubino - Memory vs. JB Asano - Rumor	3,944	0,014	Yes
Kerubino - Memory vs. Opal - Julius	3,866	0,018	Yes
Memory - Julius vs. Opal - Rumor	3,853	0,019	Yes
Rumor - Julius vs. JB Asano - Rumor	3,84	0,02	Yes
Rumor - Julius vs. Opal - Julius	3,763	0,025	Yes
Location			
Location 1 vs. Location 3	5,013	<0,001	Yes
Location 2 vs. Location 3	4,123	<0,001	Yes
Location 1 vs. Location 2	0,89	0,375	No
Ferilisation level			
High vs. Low	3,845	<0,001	Yes
Medium vs. Low	3,001	0,007	Yes
High vs. Medium	0,844	0,4	No
Ferilisation level within Location 1			
High vs. Low	4,499	<0,001	Yes
Medium vs. Low	3,626	<0,001	Yes
High vs. Medium	0,873	0,384	No
Ferilisation level within Location 2			
Medium vs. Low	1,094	0,621	No
Medium vs. High	1,085	0,481	No
High vs. Low	0,00882	0,993	No
Ferilisation level within Location 3			
High vs. Medium	1,5	0,355	No
High vs. Low	1,359	0,322	No

Low vs. Medium	0,141	0,888	No
Location within low level			
Location 2 vs. Location 3	2,135	0,101	No
Location 2 vs. Location 1	1,888	0,119	No
Location 1 vs. Location 3	0,247	0,805	No
Location within medium level			
Location 1 vs. Location 3	4,014	<0,001	Yes
Location 2 vs. Location 3	3,37	0,002	Yes
Location 1 vs. Location 2	0,644	0,521	No
Location within high level			
Location 1 vs. Location 3	3,388	0,003	Yes
Location 1 vs. Location 2	2,603	0,021	Yes
Location 2 vs. Location 3	0,785	0,434	No

[a] df degrees of freedom

[b] $p > 0.05$, not significant

[c] $p < 0.05$, significant

* For genotype: the non-significant comparisons are not mentioned in the table due to the large number of samples.

3.3. Evaluation of GPT parameters, GMP subunits concentrations and Extensograph parameters, of wheat flour samples and their blends

To get a clarification of the blending effect, GPT parameters, GMP subunits concentrations and Extensograph parameters, of pure flour samples and their blends were evaluated.

The ranges of GPT parameters (MT, PMT, AM and PM) over three locations of growth and at three levels of fertilisation of pure and blend flour samples are shown in Table 1. MT, PMT and PM parameters of pure and blend flour samples displayed a wide range of variation by locations and fertilisation levels, while AM varied slightly between levels of fertilisation. However, in location 2 and at low level of fertilisation all parameters presented the highest ranges (60 – 82 BU, 59 – 78 BU (MT); 26 – 31 BU, 24 – 30 BU (AM); 50 – 63 BU, 47 – 59 BU (PM) for pure and blend flour samples, respectively), except PMT (34 – 70 s (pure samples); 44 – 73 s (blend samples)) which showed in contrast the lowest values. That means the investigated flour samples originating from this location and at low fertilisation level are characterized by the highest and fastest build-up of the gluten network. In addition to that the high AM values obtained indicate the high elasticity of the gluten networks formed of these flour samples (Bouachra et al., 2017). Among the pure flour samples tested, Julius and Opal showed the highest values of MT, AM and PM and the lowest values of PMT over the three

locations and fertilisation levels (in location 1 at high level, in location 2 at low level and in location 3 at medium level) (data not shown). Regarding blend flour samples, the highest values of MT, PMT and PM parameters and the lowest values of PMT in location 1 were obtained for all blends at high fertilisation level, except Kerubino – Rumor and Kerubino – Memory blends. In location 2 and 3, the GPT parameter AM varied over the fertilisation levels according to each blend, whereas MT, and PM displayed the highest values and the lowest PMT values at low level (location 2) and at high level (location 3) (data not shown). These outcomes indicated the impact of genotype, location, fertilisation and their interactions on the GPT parameters of pure and blend flour samples.

The ranges of GMP subunits (GMP, HMW, LMW, LMW/HMW ratio) over three locations of growth and at three levels of fertilisation of pure and blend flour samples are also shown in Table 1. Location 2 presented the highest ranges of GMP (5.11 – 9.57 mg/g), HMW (0.69 – 1.33 mg/g), LMW (4.52 – 8.40 mg/g) and the lowest LMW/HMW ratio range (6.09 – 7.01 mg/g) at low fertilisation level for pure flour samples. In the other locations the GMP subunit ranges varied according to each genotype over the three locations and fertilisation levels. From the pure flour samples tested, Memory showed the highest values of GMP (in location 1 at high fertilisation level (9.24 mg/g), in location 2 and 3 at low level (9.57 and 5.80 mg/g, respectively), HMW (at low level over the three locations, 1.55, 1.33 and 0.74 mg/g, respectively), LMW concentrations (in location 1 at high fertilisation level (7.98 mg/g), in location 2 and 3 at low level (8.40 and 5.22 mg/g, respectively)) and the lowest LMW/HMW ratio (at medium level over the three locations), while Julius displayed the lowest GMP concentrations over the three locations. Concerning blend flour samples, all blends except Kerubino – Rumor and Kerubino – Julius had the highest GMP, HMW, LMW concentrations in location 1 at low level. In location 2 and 3, the GMP subunits concentrations depended on each genotype over the three locations and fertilisation levels. The decrease of LMW/HMW ratio of flour samples while their GMP concentration increases can be explained by the outcome of Wieser and Zimmermann (2000), Jin et al. (2011), Dhaka and Khatkar (2014) who suggested that HMW plays a positive role in dough rheological properties by contributing more to dough strength than the LMW. Besides, the differences between the observed and the calculated GMP concentrations of the wheat blends were calculated following the same method used to calculate the BEV and are illustrated in Fig. 3. The differences are considered significant when their absolute values are equal to or greater than 1.1 mg/g, which is the standard deviation as reported by Thanhaueser et al. (2014). The results of all blends are exhibited according to the three locations of growth and the three fertilisation

levels. Between all blend flour samples tested (n = 135), only 67 blends showed a significant difference between the observed and the calculated GMP concentrations over the three locations, in such a way that the highest number of blends showing this difference was acquired over the three fertilisation levels in location 3 (n = 36) in comparison with location 2 (n = 24) and location 1 (n = 7), and over the three locations at medium and high fertilisation level (n = 25; n = 24, respectively). Opal – Memory blend presents the highest difference (3.89 mg/g) in location 3 at medium level, followed by Kerubino – JB Asano in location 2 at high level (3.63 mg/g) and in location 3 at high level (3.56 mg/g). The highest differences values were mostly obtained at high or medium level, while the lowest values were achieved at low fertilisation level. Those results can be explained by the effect of location and level of fertilisation on the difference between the observed and the calculated GMP concentrations but didn't have any relationship with the variation of the BEV.

Table1 summarizes also the ranges of the Extensograph parameters (Resistance to extension, Extensibility, Energy and ratio) at 45, 90 and 135 min proving time, over three locations and at three levels of fertilisation of pure and blend flour samples. The parameters at the three proving time were fluctuating differently over the three fertilisation levels over locations of pure and blend samples, with some exceptions, in which the parameters achieved the highest values in a specific fertilisation level. For example, at 45 min proving time the R of pure samples reached the highest values in location 2 at medium level and ranged from 211 BU to 316 BU. In the same location and also at 45 min the highest values of E were obtained at low and high fertilisation levels (142 – 157 mm), whereas at 90 min proving time the high E values were achieved at low level (121 – 157 mm), and in location 3 at high level (128 – 179 mm). The En values of blend flour samples at 45 min proving time increased from low to high fertilisation level in location 1 (58 - 86 cm^2 (low level), 66 – 103 cm^2 (medium level) and 76 – 108 cm^2 (high level)). The same observation was done at 135 min proving time for E parameter, which increased in location 3 with the increase of the nitrogen fertilisation level.

As reference to the ratio R/E of blend flour samples, at 45 min proving time and in location 3 the ratio decreased as the fertilisation level increased.

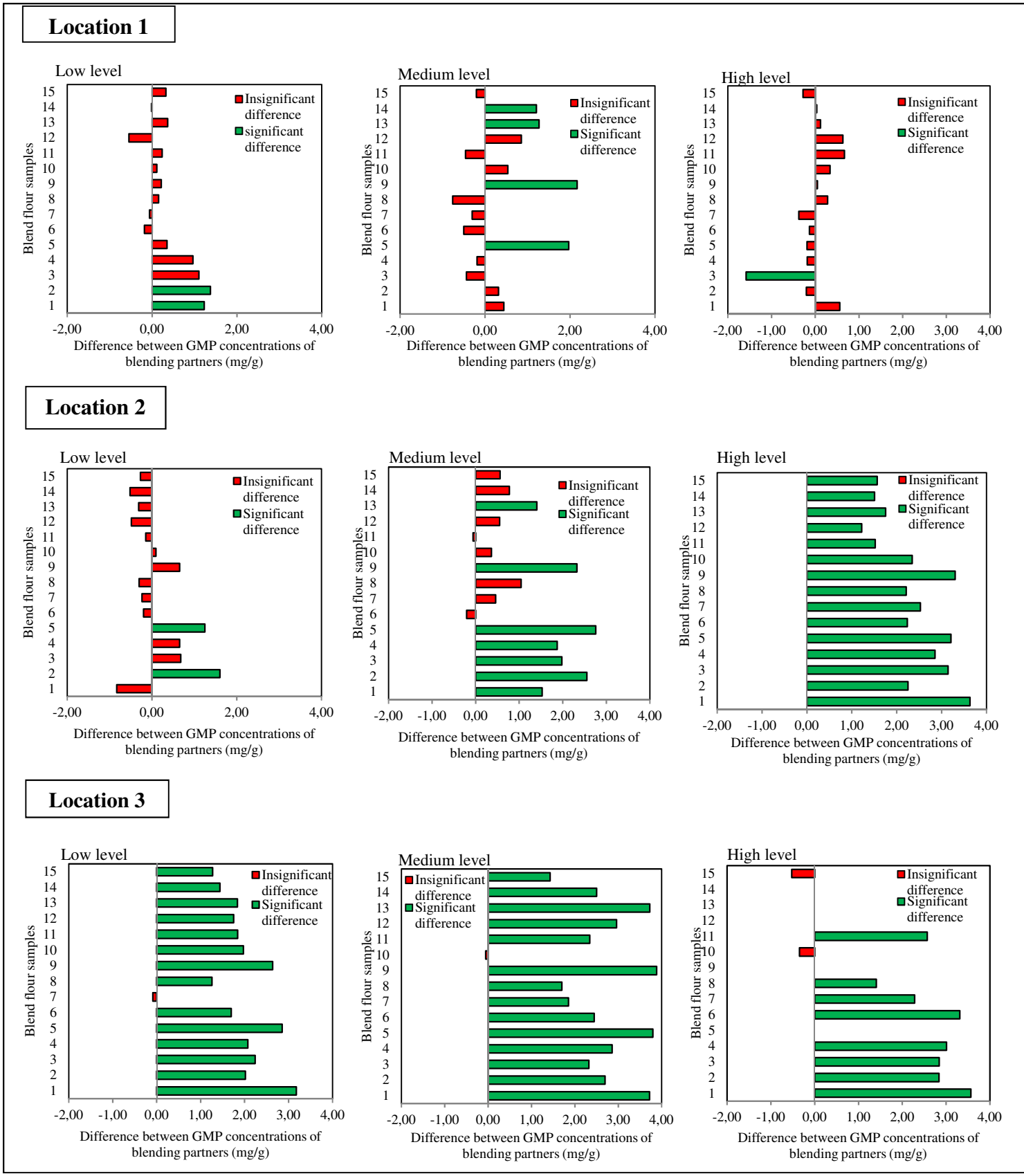


Fig. 3. Difference between GMP concentrations of blending partners of blend flour samples, from three locations and at three fertilisation levels (low, medium and high), (1.1 mg/g < Significant difference < -1.1 mg/g; -1.1 mg/g ≤ Insignificant difference ≤ 1.1 mg/g). Blend flour samples, 1: Kerubino - JB Asano, 2 : Kerubino - Rumor, 3 : Kerubino - Julius, 4 : Kerubino - Opal, 5 : Kerubino - Memory, 6 : Opal - JB Asano, 7 : Opal - Rumor, 8 : Opal - Julius, 9 : Opal - Memory, 10 : JB Asano - Rumor, 11 : JB Asano - Julius, 12 : JB Asano - Memory, 13 : Memory - Rumor, 14 : Memory - Julius, 15 : Rumor – Julius.

With pure and blend flour samples we remark that all Extensograph parameters increased with each proving time excluding En parameter, which became smaller after the third proving period at some fertilisation levels in the 3 locations; at low and high levels in location 1 and 3, and at medium level in location 2 for pure flour samples (at medium and high levels in location 1, at low and medium levels in location 2 and at low and high levels in location 3 for blends). In location 1, E parameter of pure samples decreased with each proving time, inducing the increase of R/E ratio from the low to the high level.

Those findings are in contrast with the conclusion of Šarčević et al. (2014), who investigated 19 wheat samples grown in eight environments at high and low nitrogen fertilisation levels and deduced that higher reductions of rheological parameters mean values were observed due to lower fertilisation level. This difference between our results and the outcome of this study may be the effect of the different characteristics of the plant material, the different conditions of experiment, as well as, the different fertilisation strategies used in both studies. In our study, the plant material investigated consists of pure flour samples, additionally blend flour samples, from three locations with three fertilisation levels instead of two levels (low and high). Furthermore, the Extensograph parameters taken are from the three proving times (45 min, 90 min and 135 min).

3.4. Prediction of blending effect value and loaf volume of blends via GPT parameters, GMP subunits concentrations and Extensograph parameters through different fertilisation level

Since the prediction of blending effect has not been previously investigated, there is not yet a specific method to predict the BEV and the loaf volume of blends. To optimise work time and costs, the methods applied in our study are based on the quality characteristics of the blending partners, that means GPT parameters, GMP subunits concentrations and Extensograph parameters of the two wheat flour samples involved to make each blend. Two calculation procedures were performed. The first method is based on the mean values of the parameters considered of the two flour samples forming the blend, while the second method involves the absolute difference between the two flour samples results forming the blend. The second method was taken because of the study of Bolling (1968), who suggested that the greater the difference between the rheological dough properties of the flour samples forming the blend, the greater blending effect value is obtained.

A linear Pearson correlation (r) was conducted between GPT parameters, GMP subunits concentrations, Extensograph parameters, BEV and the observed loaf volume of all blends, as

well as for each fertilisation level using the mean values $\bar{X}$ (method 1) and the difference between the parameters results of the blends partners Δ (method 2). The outcomes are summarized in Table 3.

With the first method, the highest correlation for all blends was achieved between BEV and GMP subunits concentrations ($GMP_{\bar{X}}$: r = 0.31, p < 0.001; $LMW_{\bar{X}}$: r = 0.32, p < 0.001), while the highest correlation with the second method was obtained with Extensograph parameters at 135 min proving time (ΔR (135): r = 0.44, p < 0.001), but these relationships remain weak. With both methods, no correlation was obtained between BEV, protein content, sedimentation value and GPT parameters.

At low fertilisation level, the highest correlation was found between BEV and ΔR (135min) (r = 0.45, p < 0.001) and Δ(R/E) ratio at 45 min proving time (r = 0.44, p < 0.001). While blends flour samples at medium fertilisation level showed the highest correlation between BEV and $(R/E)_{\bar{X}}$ ratio at 135 min and Δ(LMW/HMW) ratio (r = 0.50, p < 0.001). In contrast with low and medium fertilisation levels, a significant relationship was obtained at high level between BEV and ΔHMW (r = 0.65, p < 0.001). While the relationship between Extensograph parameters and BEV is still low. These results didn't confirm the outcome of Bolling (1984b), who suggested that a high difference between the extensibility values of the blending partners leads to a high blending effect. In addition to that the analyse of the variation of BEV of all blends showing a positive blending effect according to the ΔE values (data not shown) didn't fit with this suggestion to. The difference between our outcome and the results of this study may be due to the presence of just 18 blend flour samples that have a positive blending effect value ±15 ml / 100g of flour among 135 blend flour samples. Moreover Bolling (1968) didn't investigate blend flour samples with negative BEV, which is the case in our study.

Since the highest and significant correlation obtained between BEV and ΔHMW (also with $GMP_{\bar{X}}$: r = 0.59, p < 0.001) was only achieved at high fertilisation level, and these correlations increased from low to high fertilisation level (except $HMW_{\bar{X}}$ and ΔGMP at medium level) it seems that the fertilisation level plays an important role in the ability of the GMP subunits concentrations to predict BEV. That can be explained by the large impact of nitrogen fertilisation on the GMP and HMW concentrations, so that nitrogen enhances HMW synthesis and improves HMW concentration, which is closely correlated with GMP concentration as reported by Yue et al. (2007).

Table 3 Coefficients of correlation (r) and level of significance (p) between protein content, sedimentation value, GPT parameters (PMT, MT, AM, PM), GMP subunits concentrations (GMP, HMW, LMW, LMW/HMW ratio) and Extensograph parameters at the three proving time 45 min, 90 min and 135 min (En, R, E, R/E ratio) calculated by means of two methods ($\bar{X}$ and Δ) and blending effect value for blend flour samples, n = 135, and at different fertilisation levels (low, n = 45; medium, n = 45; high, n = 45).

Parameters			PC [a] (% DM)	SV [b] (ml)	PMT (s)	MT (BU)	AM (BU)	PM (BU)	GMP (mg/g)	HMW (mg/g)	LMW (mg/g)	LMW/HMW	En (cm^2) (45 min)	R (BU) (45 min)	E (mm) (45 min)	R/E ratio (45 min)	En (cm^2) (90 min)	R (BU) (90 min)	E (mm) (90 min)	R/E ratio (90 min)	En (cm^2) (135 min)	R (BU) (135 min)	E (mm) (135 min)	R/E ratio (135 min)
BEV of blend flour samples (ml/100g) (all levels, n = 135)	Methode 1 ($\bar{X}$)	r	-0.03	-0.04	-0.01	0.05	0.12	0.02	0.31	0.24	0.32	0.03	0.04	0.17	-0.15	0.18	-0.05	0.16	-0.15	0.18	-0.14	0.18	-0.19	0.11
		P [c]	n.s	n.s	n.s	n.s	n.s	n.s	<0.001	0.006	<0.001	n.s	n.s	0.05	n.s	0.04	n.s	n.s	n.s	0.04	n.s	0.05	0.03	n.s
	Methode 2 (Δ)	r	-0.07	0.03	0.16	0.02	0.00	-0.03	0.32	0.34	0.34	0.23	0.01	0.27	-0.01	0.27	-0.10	0.35	0.24	0.37	-0.22	0.44	-0.01	0.40
		p	n.s	n.s	n.s	n.s	n.s	n.s	<0.001	<0.001	<0.001	0.009	n.s	0.002	n.s	0.002	n.s	<0.001	0.006	<0.001	0.01	<0.001	n.s	<0.001
BEV of blend flour samples (ml/100g) (low level, n = 45)	Methode 1 ($\bar{X}$)	r	0.10	0.18	-0.11	0.16	0.25	0.14	0.29	0.30	0.29	-0.16	-0.03	0.11	-0.08	0.12	0.14	0.10	0.09	0.05	-0.08	0.06	-0.06	-0.01
		p	n.s	n.s	n.s	n.s	n.s	n.s	n.s	0.05	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
	Methode 2 (Δ)	r	-0.03	0.24	0.30	0.11	0.13	0.13	0.32	0.26	0.34	-0.10	0.13	0.43	0.23	0.44	-0.11	0.31	0.37	0.37	-0.14	0.45	0.34	0.42
		p	n.s	n.s	0.05	n.s	n.s	n.s	0.03	n.s	0.02	n.s	n.s	0.004	n.s	0.002	n.s	0.04	0.01	0.01	n.s	0.004	0.03	0.006
BEV of blend flour samples (ml/100g) (medium level, n = 45)	Methode 1 ($\bar{X}$)	r	-0.47	-0.31	0.26	-0.27	-0.07	-0.32	0.37	0.16	0.39	0.20	0.12	0.34	-0.42	0.37	-0.11	0.40	-0.39	0.42	-0.24	0.48	-0.47	0.50
		p	0.001	0.04	n.s	n.s	n.s	0.03	0.01	n.s	0.009	n.s	n.s	0.02	0.004	0.01	n.s	0.007	0.008	0.004	n.s	<0.001	0.001	<0.001
	Methode 2 (Δ)	r	-0.06	-0.09	0.11	-0.07	-0.15	-0.19	0.14	0.38	0.19	0.50	-0.09	-0.02	-0.34	-0.03	-0.04	0.17	0.08	0.26	-0.17	0.31	-0.26	0.34
		p	n.s	n.s	n.s	n.s	n.s	n.s	n.s	0,009	n.s	<0.001	n.s	n.s	0,02	n.s	n.s	n.s	n.s	n.s	n.s	0,04	n.s	0,02
BEV of blend flour samples (ml/100g)	Methode 1 ($\bar{X}$)	r	-0.35	-0.26	-0.11	0.17	-0.02	0.15	0.59	0.58	0.58	-0.34	-0.01	0.19	-0.25	0.22	-0.17	0.27	-0.33	0.30	-0.19	0.35	-0.36	0.36
		p	0.02	n.s	n.s	n.s	n.s	n.s	<0.001	<0.001	<0.001	0.03	n.s	n.s	n.s	n.s	n.s	n.s	0.03	0.05	n.s	0.02	0.01	0.01

(high level, n = 45)	Methode 2 (Δ)	r	-0.12	-0.08	0.07	0.02	0.00	-0.01	0.60	0.65	0.58	-0.30	-0.11	0.33	-0.05	0.33	-0.22	0.52	0.27	0.50	-0.41	0.51	0.32	0.50
		p	n.s	n.s	n.s	n.s	n.s	n.s	<0.001	<0.001	<0.001	n.s	n.s	0.03	n.s	0.03	n.s	<0.001	n.s	<0.001	0.006	<0.001	0.03	<0.001
LV of blend flour samples (ml/100g) (all levels, n = 135)	Methode 1 ($\bar{X}$)	r	0.33	0.18	0.004	-0.004	0.42	0.004	0.41	0.18	0.45	0.26	0.48	0.66	-0.34	0.59	0.26	0.44	-0.31	0.39	0.05	0.32	-0.28	0.20
		p	<0.001	0.05	n.s	n.s	<0.001	n.s	<0.001	0.04	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	n.s	<0.001	0.002	0.02
	Methode 2 (Δ)	r	0.09	0.04	-0.18	-0.12	-0.04	-0.08	0.10	0.14	0.09	-0.16	-0.43	-0.27	-0.05	-0.13	-0.25	-0.16	0.005	-0.03	-0.22	-0.071	-0.34	-0.008
		p	n.s	n.s	0.034	n.s	n.s	n.s	n.s	n.s	n.s	n.s	<0.001	0.002	n.s	n.s	0.004	n.s	n.s	n.s	0.015	n.s	<0.001	n.s
LV of blend flour samples (ml/100g) (low level, n = 45)	Methode 1 ($\bar{X}$)	r	0.61	0.48	-0.15	0.28	0.49	0.28	0.62	0.46	0.64	0.13	0.51	0.67	-0.25	0.57	0.35	0.30	-0.03	0.18	0.23	0.17	-0.01	0.00
		p	<0.001	<0.001	n.s	n.s	<0.001	n.s	<0.001	0.002	<0.001	n.s	<0.001	<0.001	n.s	<0.001	0.02	0.05	n.s	n.s	n.s	n.s	n.s	n.s
	Methode 2 (Δ)	r	0.25	0.27	0.08	0.06	0.09	0.14	0.04	0.10	0.02	-0.47	-0.34	-0.16	0.04	-0.05	-0.14	-0.07	0.09	0.00	-0.04	0.07	0.20	0.15
		p	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	0.001	0.02	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
LV of blend flour samples (ml/100g) (medium level, n = 45)	Methode 1 ($\bar{X}$)	r	0.01	-0.15	0.19	-0.40	0.37	-0.37	0.42	0.09	0.46	0.32	0.47	0.75	-0.58	0.73	0.14	0.62	-0.53	0.59	-0.16	0.55	-0.56	0.54
		p	n.s	n.s	n.s	0.007	0.01	0.01	0.004	n.s	0.002	0.04	0.001	<0.001	<0.001	<0.001	n.s	<0.001	<0.001	<0.001	n.s	<0.001	<0.001	<0.001
	Methode 2 (Δ)	r	0.02	-0.20	-0.42	-0.30	-0.23	-0.28	0.15	0.26	0.09	-0.12	-0.60	-0.41	-0.29	-0.29	-0.15	-0.44	-0.13	-0.22	-0.15	-0.35	-0.62	-0.25
		p	n.s	n.s	0.004	0.05	n.s	n.s	n.s	n.s	n.s	n.s	<0.001	0.005	<0.001	0.05	n.s	0.002	n.s	n.s	n.s	0.02	<0.001	n.s
LV of blend flour samples (ml/100g) (high level, n = 45)	Methode 1 ($\bar{X}$)	r	-0.14	-0.05	0.15	-0.07	0.22	-0.02	0.39	0.18	0.43	0.20	0.47	0.74	-0.50	0.68	0.34	0.68	-0.61	0.64	0.18	0.63	-0.57	0.58
		p	n.s	n.s	n.s	n.s	n.s	n.s	0.01	n.s	0.005	n.s	0.002	<0.001	<0.001	<0.001	0.03	<0.001	<0.001	<0.001	n.s	<0.001	<0.001	<0.001
	Methode 2 (Δ)	r	-0.03	0.06	-0.02	0.02	0.12	0.06	0.24	0.27	0.23	-0.26	-0.61	-0.31	-0.01	-0.09	-0.66	0.02	0.12	0.18	-0.53	0.06	0.08	0.18
		p	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	<0.001	0.05	n.s	n.s	<0.001	n.s	n.s	n.s	<0.001	n.s	n.s	n.s

[a] Crude Protein content (% DM).

[b] Sedimentation value (ml).

[c] $p > 0.05$, not significant (n.s).

As can be seen from the r values of the correlations for BEV, none of the parameters studied produced a high significant correlation with BEV over all fertilisation levels. For that reason, best subset regression analysis was made, to develop a model enabling a better prediction of BEV. Protein content, sedimentation value, ΔGMP subunits concentrations and all Δ Extensograph parameters from the three proving times were included in the analysis as independent variables to predict BEV (dependant variable) of all blends from the three fertilisation levels (Table 4). The first model (model 1) produced had a very low R^2 (R^2 = 0.18) and the addition of other variables (ΔHMW in model 2; ΔHMW and ΔLMW/HMW in model 3) lead to only minor increases in R^2 (R^2 = 0.20, model 2 and R^2 = 0.23, model 3) and to a high decrease in F value from 30.9 (model 1) to 15.6 (model 2) and 12.3 (model 3). This decrease indicates a decrease in the predictive capability of these models, which is probably due to overfitting regression models.

The Extensograph parameters (En, R, E R/E ratio) even in combination with GMP subunits concentrations (GMP, HMW, LMW, LMW/HMW ratio), protein content and sedimentation value are not good predictors of BEV of blend flour samples from different fertilisation levels. The relationship between these parameters and BEV remains dependent on the level of fertilisation, genotype, location and their interactions.

Another important factor was considered to predict the blending effect which is loaf volume of blends. And to investigate the parameter that can predict it, a linear Pearson correlation was performed between GPT parameters, GMP subunits concentrations, Extensograph parameters and the observed loaf volume of all blends, as well as for each fertilisation level always using the mean values $\bar{X}$ and the difference between the parameters results of the blends partners Δ (Table 3).

Among all parameters studied, $R_{\bar{X}}$ (45min) showed the highest and significant correlation with loaf volume of all blends ($r = 0.66$, $p < 0.001$), while GPT parameters and GMP subunits concentrations didn't display a significant relation to loaf volume. Over the three fertilisation levels loaf volume was significantly related to $R_{\bar{X}}$ (45min), $r = 0.67$, $p < 0.001$ at low level, $r = 0.75$, $p < 0.001$at medium level and $r = 0.74$, $p < 0.001$ at high level. This correlation was more stable against nitrogen fertilisation increase from medium to high level, than from low to medium level. The Extensograph parameter $(R/E)_{\bar{X}}$ ratio at 45 min proving time displayed also a good correlation with loaf volume over the three level of fertilisation ($r = 0.57$, $p < 0.001$ at low level, $r = 0.73$, $p < 0.001$at medium level and $r = 0.68$, $p < 0.001$ at high level). These results are in line with the outcome of Ktenioudaki et al. (2011), who correlated loaf volume with dough rheological parameters, and found out a significant relationship. However,

there was no significant correlation between loaf volume and GPT parameters. Among GMP subunits, $GMP_{\bar{X}}$ and $LMW_{\bar{X}}$ at low level showed a significant correlation to loaf volume ($r = 0.62$, $p < 0.001$ and $r = 0.64$, $p < 0.001$, respectively), and became not significant with the increase of nitrogen fertilisation level, which is in contrast with the results of Yue et al. (2007). That might be due to the impact of the environmental factors, such as location, genotype, and their interaction with the nitrogen fertilisation level as reported by Triboi et al. (2000). Besides GMP concentration, particle size is also an important factor in determining wheat baking quality, and the effect of fertilisation level on it also can influence our results.

Best subset regression analysis with loaf volume as the dependent variable and the other parameters (protein content, sedimentation value, $En_{\bar{X}}$, $R_{\bar{X}}$, $E_{\bar{X}}$ and $(R/E)_{\bar{X}}$ Extensograph parameters at 45 min proving time) as independent variables was carried out to highlight the best predictors for blends loaf volume (Table 4). In combination with $R_{\bar{X}}$ (45 min), protein content and sedimentation value turned out to be good predictors for blends loaf volume ($R^2 = 0.67$), with a significant F value of 86.2 ($p < 0.001$) (Model 3, Table 4), but the best prediction was achieved with model 4 using $GMP_{\bar{X}}$ in addition to $R_{\bar{X}}$ (45 min), protein content and sedimentation value ($R^2 = 0.69$). This model can provide an explanation of loaf volume variation by 69% with an uncertainty of ± 23 ml, which is better than the uncertainties of the other models.

To test if our results can be more generalizable and that the model will fit other samples, a cross validation was carried out partitioning our data according to each fertilisation level. This resulted in good correlation between observed and predicted loaf volume over the three fertilisation levels ($r = 0.89$, $p < 0.001$ at low level; $r = 0.84$, $p < 0.001$ at medium level and $r = 0.83$, $p< 0.001$ at high level) (Fig. 4).

Those results point out that the model created involving the mean values of the blend partners of the followed parameters: protein content, sedimentation value, GMP concentration and the Extensograph parameter R (45 min), can be useful to explain variation in loaf volume of blends whichever is the level of nitrogen fertilisation (low, medium or high), and subsequently predict the BEV that is normally noticed only by means of the baking tests.

Table 4: Prediction of blending effect value and loaf volume of blend flour samples using best subsets regressions of protein content, sedimentation value, GMP subunits concentrations and Extensograph parameters.

Dependant variable	Model	n	Factors	r	RSQ [a]	SEE [b]	F	p
BEV [c]	1	135	ΔR (135 min)	0,42	0,18	21	30,9	<0,001
	2	135	ΔR (135 min) + ΔHMW	0,45	0,20	21	15,6	<0,001
	3	135	ΔR (135 min) + ΔHMW + ΔLMWHMW	0,48	0,23	21	12,3	<0,001
LV [d]	1	135	$R_{\bar{X}}$ (45 min)	0,66	0,44	30	99,6	<0,001
	2	135	$R_{\bar{X}}$ (45 min) + PC [e]	0,81	0,66	24	122,8	<0,001
	3	135	$R_{\bar{X}}$ (45 min) + PC + SV [f]	0,82	0,67	23	86,2	<0,001
	4	135	$R_{\bar{X}}$ (45 min) + PC + SV + $GMP_{\bar{X}}$	0,83	0,69	23	69,8	<0,001

[a] Coefficient of determination (R^2).

[b] Standard Error of Estimate.

[c] Blending effect value (ml/100g of flour).

[d] Loaf volume (ml/100g of flour).

[e] Crude Protein content (% DM).

[f] Sedimentation value (ml).

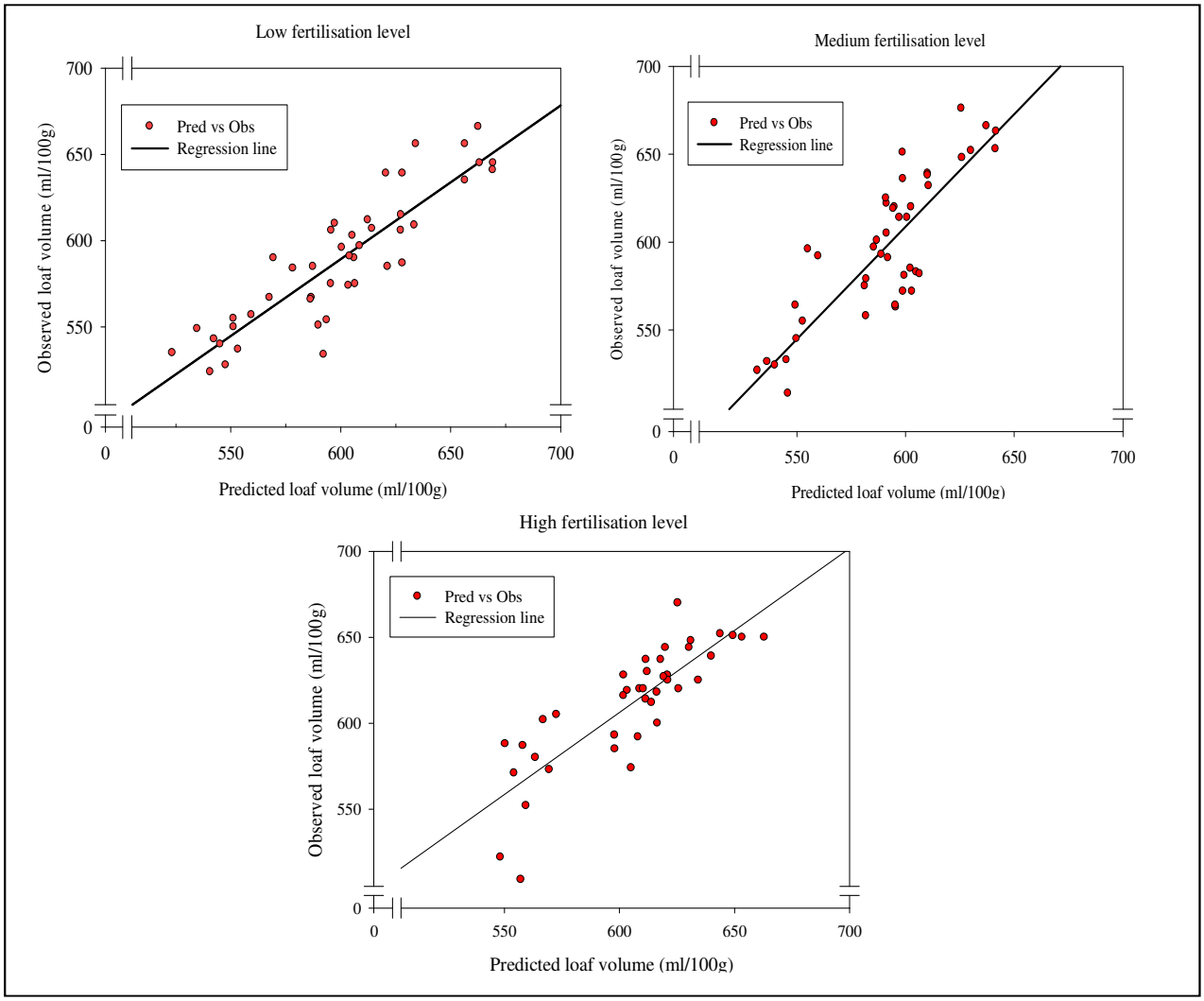


Figure 4: Scatter diagram indicating the relationship between the observed and predicted loaf volume (Regression equation: Loaf volume = 65,507 + (12,661 * Protein content) + (1,614 * Sedimentation value) + (5,278 * GMP) + (1,055 * R (45min)), for blend flour samples at different fertilisation levels (low, n = 45; medium, n = 45; high, n = 45).

Conclusions

The current study helped us in understanding a number of phenomena related to blending effect, which is not completely understood, but could be a good alternative to predict the loaf volume of soft wheat blends. The blending effect which can be positive, negative or insignificant is influenced by genotype, location, fertilisation level and the interaction location × fertilisation level. However no significant difference was obtained between high and medium fertilisation level over the locations considered, thus implying that the late season fertilisation doesn't affect the blending effect value and the reduction of nitrogen fertilisation from high to medium level is possible. However, fertilisation level remains a crucial source of variation of the relationship between GPT, GMP and Extensograph parameters calculated on the basis of the quality characteristics of the blending wheat flour partners, and the blending effect value, which makes its prediction by means of those parameters, even in combination, not possible. Among these parameters, $R_{\bar{X}}$ (45min) showed the highest, significant and environmental stable correlation with blends loaf volume. A linear function of $R_{\bar{X}}$ (45 min),$GMP_{\bar{X}}$, protein content and sedimentation value can provide an explanation of blends loaf volume variation by 69% with an uncertainty of ± 23 ml, whichever is the level of nitrogen fertilisation.

Further studies are necessary to strengthen these results and investigate the blending effect, considered as a new approach to increase the baking quality of soft wheat varieties and to reduce nitrogen fertilisation level.

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III. Discussion and conclusions

As previously mentioned, by blend flour samples a synergistic effect, known as blending effect, is observed when the loaf volume of flour blend exceeds or is inferior to the loaf volume mathematically assumed from the wheat flours blended loaf volumes. Consequently, from this effect a major advantage might be benefited, which is an improvement of the blend loaf volume while reducing nitrogen fertilisation for economic and ecological aims. Therefore, before the assessment of blend baking quality and the prediction of loaf volume, the assessment of BEV was carried out taking into account the impact of genotype, location of growth and the level of late nitrogen fertilisation.

As reported in the present study, it seems that location, genotype, level of fertilisation and their interaction have a large influence on the BEV. But the high late fertilisation level doesn't have an impact on the BEV since the highest values are achieved at medium fertilisation level. This outcome was confirmed by the ANOVA analysis which demonstrated that a high amount of late nitrogen fertilisation doesn't apply automatically a positive and high BEV, since medium fertilisation level can lead to the same blending effect achieved with high level. Moreover, the interactions genotype × fertilisation level and genotype × location do not impact the BEV, in contrast with the interaction location × fertilisation level, which was only observed in some locations between low and high level and between low and medium level. Apropos medium vs. high, any significant difference was obtained over the all locations. This outcome approves our previous observation and validates that a reduced late nitrogen fertilisation from high to medium level doesn't impact the BEV and the same result can be achieved. A comparison of these results with previous studies is not conceivable, since because the effect of genotype, location and fertilisation level on blending effect has not been previously investigated.

To investigate the relationship between GPT parameters, GMP and its subunits concentration, Extensograph parameters and the BEV two methods were applied, by reason of the prediction of blending effect has not been previously investigated, and there is not yet a specific method to predict the BEV and the loaf volume of blends.

The first method is based on the mean values of the parameters considered (GPT parameters, GMP subunits concentrations and Extensograph parameters) of the two flour samples forming the blend (method 1:$\overline{X}$), while the second method involves the absolute difference between the two flour samples results forming the blend (method 2: Δ).

The correlation conducted between the BEV and the parameters investigated demonstrated that with the second method, the highest and significant correlation was only achieved at high fertilisation level and was obtained between BEV and ΔHMW ($r = 0.65$, $p < 0.001$, which highlighted the role of the fertilisation level in the ability of the GMP subunits concentrations to predict BEV. That can be explained by the large impact of nitrogen fertilisation on the HMW synthesis, which is closely correlated with GMP concentration (Yue et al., 2007). However, the correlation between$GMP_{\overline{X}}$, $LMW_{\overline{X}}$ and loaf volume which is significant at low level ($r = 0.62$, $p < 0.001$ and $r = 0.64$, $p < 0.001$, respectively), became not significant with the increase of nitrogen fertilisation level. That might be due to the impact of the environmental factors, such as location, genotype, and their interaction with the nitrogen fertilisation level as reported by Triboi et al. (2000), which exceeds the effect of the increase of fertilisation level on GMP. Moreover, particle size of the GMP subunits is also an important factor in determining wheat baking quality, and the effect of fertilisation level on it also can influence our outcome.

The relationship between Extensograph parameters and BEV, calculated with the second method, remains lower in comparison with the correlation between $GMP_{\overline{X}}$, ΔHMW and BEV. These results didn't confirm the outcome of Bolling (1984b), who suggested that a high difference between the extensibility values of the blending partners leads to a high blending effect. The difference between our outcome and the results of this study may be due to the presence of just 18 blend flour samples that have a positive blending effect value ±15 ml / 100g of flour among 135 blend flour samples. Moreover Bolling (1968) didn't investigate blend flour samples with negative BEV, which is the case in our study.

However, the Extensograph parameter $R_{\overline{X}}$ (45min) showed the highest and significant correlation with loaf volume of all blends ($r = 0.66$, $p < 0.001$), this relationship remained also significant over all fertilisation levels. These results are in accordance with the outcome of Ktenioudaki et al. (2011), who demonstrated a significant relationship between loaf volume and dough rheological parameters. GPT parameters didn't display a significant relation to loaf volume.

Since none of the parameters studied produced a high significant correlation with BEV over all fertilisation levels, a best subset regression analysis was performed with BEV of all blends from the three fertilisation levels as dependant variable and protein content, sedimentation value, ΔGMP subunits concentrations and all Δ Extensograph parameters from the three proving times were included in the analysis as independent variables, in order to develop a model enabling a better prediction of BEV.

All the models produced didn't supply a significant explanation of the variation of the BEV. The relationship between the parameters considered and BEV remains dependent on the level of fertilisation, genotype, location and their interactions.

But on the other hand, the model created with the parameter $R_{\overline{X}}$ (45 min) in combination with$GMP_{\overline{X}}$, protein content and sedimentation value can provide an explanation of loaf volume variation by 69% with an uncertainty of ± 23 ml, which is better than the uncertainties of the other produced models. This model was tested by means of a cross validation in order to determine if the results obtained can be more generalizable and that the model will fit other samples. This resulted in good correlation between observed and predicted loaf volume over the three fertilisation levels ($r = 0.89$, $p < 0.001$ at low level; $r = 0.84$, $p < 0.001$ at medium level and $r = 0.83$, $p < 0.001$ at high level).

Those results point out that the model created involving the mean values of the blend partners of the followed parameters: protein content, sedimentation value, GMP concentration and the parameter R (45 min), can be useful to explain variation in loaf volume of blends whichever is the level of nitrogen fertilisation (low, medium or high), and subsequently predict the BEV that is normally noticed only by means of the baking tests.

Chapter 6

General discussion and conclusions

1. General discussion

The assessment of wheat baking quality is based since many decades on direct methods; baking tests and on indirect methods, mainly crude protein content as it showed a linear relationship with the loaf volume. However, baking tests necessitate specific equipment of baking facilities, experienced staff, time, and a considerable quantity of wheat material. Moreover, in recent years some approved wheat varieties in Germany do not have a linear dependency between protein quantity and loaf volume due to high functionality of the gluten protein. Therefore, the classical quality parameters appear to be no longer suitable to assess the baking quality of modern soft wheat varieties.

This current thesis investigated the ability of specific methods to assess the baking quality of soft wheat flour samples in different environmental conditions, for the purpose of predicting loaf volume, and thus replace the current direct and indirect methods.

Accordingly, chapter 3 of this study looks into the ability of the GPT to achieve the objective previously determined. The GPT, as rapid shear-based method for measuring the gluten aggregation properties, has been proposed for the quick evaluation of wheat baking quality by measuring the aggregation behavior of gluten (Kaur and Seetharaman, 2011). Due to three key elements: small sample size (3-10 g), easy handling, results within some minutes (1-10 min), a lot of attention was given to this equipment, and its capacity to assess the wheat gluten quality. During the GPT test, the flour sample is mixed with the solvent according to a defined flour/solvent ratio and then subjected to intense mechanical action, due to the high speed of the mixing paddle. These conditions induce the formation of the gluten network and increase of the slurry consistency until reaching a maximum value. Then, the continuous mechanical stress causes the breakdown of the gluten network, which is recorded as a decrease in consistency (Marti et al., 2015a).

Previous studies reported that the parameters supplied significant correlation with the conventional parameters related to dough mixing stability, extensibility, and tenacity, used for wheat characterization (Marti et al., 2015a), and recommended the GPT as a valid screening tool for durum wheat quality (Marti et al., 2013, 2014). However, the available studies have not yet investigated the relationship between the GPT parameters and the loaf volume of soft wheat flour samples on the basis of multigenotype and multilocation analysis, since they have tested the GPT method either with a restricted number of samples or within a single-year analyses, which could only provide an indication of the applicability of the tested method, without taking into account the interactions over different locations of growth and the interaction of genotype-by-location. Nevertheless, genotype, location and their interaction

have an important effect on the evaluation of a new method because the various components of flour protein differ in their response to environmental and genotype influence and will change according to location, cultivating conditions and season (Kaya and Akcura, 2014). Consequently, an optimisation of the GPT standard method for a precise and universally usable application for predicting wheat quality based on a multiyear and multilocation analysis was carried out using different solvents and varying the solvent/flour ratio, whereas the temperature has been raised to 36 °C, and the mixing paddle was operated at a speed of 2750 rpm.

The solvents used are: Distilled water, 5.0 % sucrose solution (w/w), 5.0 % (w/w) sodium carbonate solution and 5.0 % (w/w) lactic acid solution. These solvents are the Solvent Retention Capacity method (SRC) solvents and were selected because of the ability of the SRC to predict the functionality of different flour constituents, by quantifying the swelling behavior of flour polymer networks and, hence, their ability to retain a given solvent (Kweon et al., 2011; Slade and Levine, 1994; Duyvejonck et al., 2011). In addition to these solvents, 1.0 % sodium chloride solution (w/w) was also used due to its ability during hydration and mixing to shield the charges on the gluten protein, reducing electrostatic repulsion between proteins and allowing them to be more closely associated, as reported by Miller and Hoseney, 2008.

Among the solvents used to run the GPT, distilled water and 5 % sucrose had an effect on flour samples, since the gluten network formation was clearly demonstrated by the torques obtained on the GPT. While 5 % sodium carbonate didn't generate any peak formation. This observation can be attributed to the effect of the pH on wheat proteins, which can induce modification of the structure of dough proteins through electrostatic attraction or repulsion resulting from changes in the degree of ionization of ionizable groups on flour proteins. As mentioned in chapter 3, a pH value of 11.6, in the presence of sodium carbonate provided strong repulsive forces between highly negative charges along gluten protein chains, which might be responsible for preventing the gluten protein molecules from associating and forming networks. However, these three solvents didn't enable a significant classification and differentiation between the different flour samples investigated based on quality classes, protein content and loaf volume.

5 % lactic acid and 1 % sodium chloride on the other hand displayed significant GPT responses and provide a good classification and a differentiation between the different flour samples on the basis of quality classes, protein content and sedimentation value. In the presence of lactic acid, the flour samples showed a rapid build-up of the gluten network which

led to a sharply defined peak followed by a rapid breakdown. These observations are characteristics commonly exhibited by weak flours with poor technological quality (Goldstein et al., 2010). This weakness is due to the low pH in the presence of lactic acid. At pH below 4.0 there was a sizable positive charge and an increase in intramolecular electrostatic repulsion enhancing gluten proteins unfolding and increasing the exposure of their hydrophobic groups, but the presence of strong intermolecular electrostatic repulsive forces prevent the formation of new bonds. Furthermore, at this low pH the proteolytic activity is further enhanced and might lead to the degradation of the gluten proteins responsible for the dough's firmness and elasticity resulting in a softer and less elastic gluten network (Clarke et al., 2004). Whereas, sodium chloride contributes to obtain a slow build-up in gluten network formation, compared to the result obtained using lactic acid. This could be explained by the assumption that salt draws away water molecules from gluten structures to interact with sodium and chloride ions, inducing a reduction in the hydration rate of the gluten, which results in the longer development time of dough during mixing, as it was shown from the previous work by McCann and Day (2013). Consequently, the results obtained from the two GPT methods using 5 % lactic acid and 1% sodium chloride were used to calculate the coefficients of correlation between the GPT parameters and loaf volume.

Using 5% lactic acid, the area A4-A5 (the area under the curve from MT to PM) presented the highest correlation with loaf volume ($r = 0.70$, $p < 0.001$), which led to the assumption that this area represents the gliadin content of wheat samples. That means a decrease in gluten strength caused by high gliadin content require a high energy to disaggregate the gluten network formed, approving that high gliadin content is negatively correlated with the gluten strength. Nevertheless, the correlation between the GPT area A4-A5 does not reach the correlation values obtained between sedimentation value, protein content and loaf volume (Chapter 3, Table 2). This correlation didn't remain stable over the different locations of growth over the two harvest years 2013 and 2014. This emphasises that different correlation coefficients between the same parameters can be expected at different locations due to the impact of location on growth on the relationship between GPT area A4-A5 and loaf volume. Moreover, this coefficient of correlation between loaf volume and GPT area A4-A5 does not reach the correlation values obtained between sedimentation value, protein content and loaf volume. Thus, the GPT using 5 % lactic acid cannot be considered as an optimal tool to replace both indirect methods: protein content and sedimentation value in the prediction of loaf volume.

In summary, the highest correlation between the loaf volume and the GPT parameters using 5 % lactic acid was always achieved with the area A4-A5, but varying environmental condition means instability in the strength of the relationship between A4-A5 and the loaf volume, which was distinctly underlined in this study. Moreover, since the relationship between protein content, sedimentation value and loaf volume remains stronger as that obtained between A4-A5 and loaf volume, the GPT using 5 % lactic acid is not sufficient to characterize and predict baking quality in bread wheat varieties.

Another GPT parameter, using 1 % sodium chloride, showed the highest correlation with loaf volume, which is AM (the torque 15 s before the PMT), ($r = 0.77$, $p < 0.001$, in the harvest year 2013 and $r = 0.63$, $p < 0.001$, in the harvest year 2014). This observation leads to the assumption that this parameter may reflect the glutenin content of wheat samples, since glutenin is able to generate a cohesive structure of the gluten network, taking into consideration that an increase of glutenin content increases gluten strength, i.e. elasticity (Melnyk et al., 2012). The high molecular weight subunits of glutenin, which are relevant to elasticity, possess a number of cysteine residues available to form intermolecular cross links. This is because the degree of cross-linking will determine the bulk elastic properties. With a low degree of cross-linking the extensibility will be high, but with increased disulfide cross-linking the material would become more rubber-like (Shewry et al., 1991). Consequently, the higher the degree of cross linking is, the higher the AM value and the gluten network elasticity we get. This elasticity is increased in the presence of sodium chloride, in a way that dough shows higher resistance to extension as reported by McCann and Day (2013). However, in the presence of a high concentration of this solvent, electrostatic forces become negligible due to kosmotropic anions and cations of the salt which have an effect on water structure, and consequently, on gluten aggregation (Zhang and Cremer, 2006). Kosmotropes reinforce hydrophobic interactions between gluten proteins due to less water availability and result in less unfolding and hydrogen bonding between unfolded chains. Subsequently more mixing time is necessary to enable the formation of interprotein interactions through hydrogen bonding, hydrophobic interactions and inter-disulfide bonding that contribute to gluten development (Kinsella and Hale, 1984), thus the formation of these interactions is delayed like in the study of Marti et al. (2015a), who rum the GPT with 0.33 mol/L sodium chloride. Therefore, in our study on account of the low sodium chloride (0.17 mol/L), the formation of the protein interactions took place in torque before maximum AM, which explain the good correlation between this parameter and the loaf volume.

Consequently, it was reported in the current study that sodium chloride in low concentrations as a solvent to perform the GPT is needed to obtain suitable GPT parameters values enabling the correlation with the loaf volume. This outcome is different from the conclusion of Marti et al. (2015a), who suggested that water is also sufficient to gain satisfactory estimates of conventional parameters like dough strength.

According to quality classes, (E; A; B), the attribution of a given sample to a specific quality class in function to protein content, sedimentation value and GPT parameters (AM using sodium chloride and A4-A5 using lactic acid) is not straightforward (Chapter 3: Figure 2a, in the presence of lactic acid; Figure 2b in the presence of sodium chloride), confirming that wheat flour quality is a complex property that varies in a continuous manner and actually relies on several physical, chemical and rheological parameters, , when taken separately, did not allow a clean separation between the various classes and whose interpretation took implicitly into account complex interactions among all the parameters themselves (Foca et al., 2007; Marti et al., 2015a,b).

The correlations between protein content or sedimentation volume and loaf volume over the different locations of growth in the two harvest years (2013 and 2014) are in most cases higher than the correlation values obtained by means of GPT (Chapter 3, Table 2). However, the correlation between the GPT parameter AM and the loaf volume are often very high and stable over different locations, indicating that GPT with sodium chloride as a solvent measures protein quality rather than differences in protein content. That means genotype by environment interaction plays an important role in the ability of the GPT method using 1 % sodium chloride to predict loaf volume, with the major environmental influenced factor being protein content. Therefore, GPT results should not be interpreted as predictors of loaf volume unless the corresponding protein content is taken into consideration. This outcome could not be highlighted without an extensive set of samples to evaluate a method, which broadens the range of possible data, and gives a better understanding of the environmental and genotype influence.

Considering the results obtained, a combination of two rapid tests is recommended to assess baking quality and predict the loaf volume of soft wheat flour samples. Consequently, among the models determined by means of a multiple regression analysis with loaf volume as the dependent variable and all indirect tests (protein content, sedimentation value and GPT area AM) as independent variables, the best prediction of loaf volume was a linear function of protein content and the GPT parameter AM (Chapter 3, Table 3). This model can explain the variation in loaf volume by 63 % and provides estimates of loaf volume within an uncertainty

of ±39 ml (an uncertainty equal to ±6.5 % at a loaf volume of 600 ml/100 g) which is better than the uncertainties of the other models created and comparable to the uncertainty of the reference method (±36 ml). The validation of this model was carried out by means of an independent set of data (21 different bread wheat varieties from the harvest year 2015 cultivated at eight different locations in Germany), due to the necessity to integrate locations and years (seasonal variation) in a method validation in order to offer a realistic idea of the predictive power on future unknown samples and the applicability in the baking industry. This resulted in 64 % correct and in 36 % incorrect predictions (Chapter 3, Figure 3).
Those results indicate that the model created, didn't fit the data very well and it cannot be expected to replace the actual measurement of loaf volume, but nevertheless it could be a useful and rapid screening test in breeding for improved baking quality in bread wheat.

That brings us to the assessment of the wheat baling quality by means of another parameter, which is the gluten macropolymer (GMP). The GMP, known also as gel protein, because it forms a gel like layer on top of the starch pellet when SDS extracts are centrifuged (Graveland et al., 1979), can be isolated by dissolving the SDS unextractable glutenin subfractions under reducing conditions (i.e, interchain disulfide bonds are broken) and subsequently, quantitated using HPLC (Cinco-Moroyoqui and MacRitchie, 2008; Cornec et al., 1994). Previous studies confirmed the importance of GMP in assessing physical dough properties and demonstrated a high correlation between GMP concentration and other parameters; like sedimentation value, dough strength, the resistance to extension (Moonen et al., 1982; MacRitchie and Lafiandra, 1997). Nevertheless, a few of the available studies enquired into the relationship between GMP and loaf volume and reported that they are closely related to each other (Thanhaeuser et al., 2014). Moreover, studies looking at this relationship on the basis of a multigenotype and multilocation analysis, in order to integrate influences of location, variety and their interaction and eventually offer a realistic idea of the estimation of the relationship between GMP and loaf volume are not yet available. However, the nature of wheat varieties (genotype) and environment (location of growth) and their interaction has a pivotal influence on the wheat baking quality for example through variations in gluten protein polymer structure (Johansson et al., 1999; Zhu and Khan, 2001).
This outcome was confirmed with the results obtained in the current study. The investigation of a large number of different soft wheat flour samples from different locations in Germany (eight locations from each harvest year; 2013 and 2014) demonstrated that flour samples from the harvest year 2013 showed higher GMP concentrations in comparison with flour samples

from 2014, which could be explained by the heat effect, attributed to the environmental conditions characterising the harvest year 2014 in Germany (Deutscher Wetterdienst, 2014). As reported by Cairns et al., 2012, increased temperature during grain filling induces disturbances by the GMP particle formation. Heat stress accelerates physiological and biochemical reactions in grains, which accelerates the rate of grain dehydration, and subsequently shortens the time of grain development (grain filling rate is accelerated). This situation slows the biosynthesis of glutenin as reported also by DuPont and Altenbach, (2003), leading to a decrease in the GMP concentration with an increase of glutenin particles. Higher temperatures and dehydration induce a more rapid and an increased disulphide bonds formation at the time of polymerisation and assembly into larger insoluble glutenin particles, as well as a fusion of glutenin particles, which cause an hyperaggregation and formation of larger particles (Don et al., 2005). This outcome was supported by the GMP concentrations of the cultivar Julius, the only one reference variety harvested over the two harvest years. We obtained a mean concentration of 3.9 mg/g for 2013, and a lower concentration for 2014 of 3.6 mg/g. The same cultivar deriving from one location and from the harvest year 2010 was investigated by Thanhaeuser et al. (2014), who obtained a lower LMW/HMW ratio than the ratio that we obtained in the current study. This difference between our outcome and the results of this study may be the effect of the environmental conditions (harvest year and location of growth) as well as the used milling device, which is a Quadrumat junior Mill from Brabender at variance with the Buehler laboratory mill MLU 202 that we used to mill the wheat grains, since the milling process affects strongly wheat flour composition and wheat quality characteristics (El-Porai et al., 2013).

The effect of quality class, genotype and location of growth on the indirect parameters (protein content and sedimentation value), loaf volume, GMP and GMP subunits concentrations was also statistically highlighted. Concerning flour samples from the harvest year 2013, wheat quality class was observed to be statistically significant source of variation ($p \leq 0.001$) for all considered parameters (Chapter 4, Table 1). This outcome is an expected result, because the German wheat classification system allocates samples to a certain quality group on the basis of many quality traits, among them crude protein content, sedimentation and loaf volume, in such a way that E-class wheat has the highest quality, followed by A-class, B-class and C-class wheat with the lowest quality, which is not useable for baking and hence not investigated in our study. Moreover, GMP fraction is associated with protein content which explained the results obtained regarding GMP, HMW, and LMW concentrations for each wheat quality class (Spiertz et al., 2006). For the same flour samples,

genotype turned out to be also a significant source of variation for all parameters examined, that means protein content, sedimentation value, loaf volume, GMP, HMW, LMW concentrations and LMW/HMW ratio, while the distinction between locations is possible only on the basis of protein content, sedimentation value and the ratio LMW/HMW. Moreover, location represented the largest source of variation for protein content ($H = 50.8$, $p \leq 0.001$) in comparison with quality class and genotype ($H = 25.7$, $p \leq 0.001$ and $H = 43.9$, $p \leq 0.001$, respectively), when genotype affected more the other measured parameters, sedimentation value, loaf volume, GMP, HMW, LMW concentrations and LMW/HMW ratio. This outcome is confirmed by the study of Johansson et al. (2001), who reported that protein concentration is determined both genetically and environmentally. In addition to that, Zhu and Khan (2001) investigated six genotypes of hard red spring wheat, grown at seven environments in North Dakota during 1998 and demonstrated that gluten macro polymer was controlled more by genetic than environmental factors, which was also highlighted in our present study.

In contrast, wheat samples from 2014 didn't present any significant difference between wheat quality classes and genotypes for GMP, HMW, LMW concentrations and LMW/HMW ratio, which could be explained by the heat effect on GMP and GMP subunits, , disallowing a differentiation between wheat quality classes on the basis of these parameters. However, a significant difference was demonstrated among locations for GMP, GMP subunits concentrations and protein content, indicating that the location of growth affected these parameters. This difference seen between wheat samples from 2013 and 2014 may be attributed to the high temperatures, affecting wheat grain in terms of various physiological and biochemical processes, and which depends on the responsiveness and sensitivity of each genotype to the environment (Iqbal et al., 2017; de Leonardis et al., 2015).

As reference to the relationship between GMP, GMP subunits concentrations, LMW/HMW ratio and loaf volume, results from this study demonstrated that all measured parameters (GMP, HMW, LMW concentrations), except LMW/HMW of wheat flour samples from 2013 showed positive correlation with loaf volume, while wheat samples from 2014 didn't show any significant relation to loaf volume. The highest coefficient of correlation obtained in 2013 was achieved with HMW concentration ($r = 0.55$, $p < 0.001$). This outcome didn't confirm the results obtained by Thanseauser et al. (2014) who obtained a close relationship between GMP fractions and loaf volume (GMP: $r = 0.80$, HMW: $r = 0.77$ and LMW: $r = 0.77$). This difference might be linked to the different baking tests applied in the two studies. Thanseauser et al. (2014) used the Micro Rapid Mix Test as a baking test with 10 g of flour with an optimised mixing of each flour to optimum consistency, instead of the Rapid Mix test that we

used in our study, which is assumed to have a large impact on the correlation between loaf volume and the GMP fractions. The Rapid Mix Test requires dough kneading of 1 min at a speed of 1400 rpm which might be capable of disintegrating the GMP-Network, in contrast with a gentle kneading which can maintains the GMP-Network, in the case of the Micro Rapid Mix Test with a mixing of 1 min at 1250 rpm. In addition to that, the differences between the two studies in the plant material preparation, like for example milling, impacts the protein content, GMP fractions and subsequently the different correlation values. Moreover, the coefficient of correlation between loaf volume and GMP fractions does not reach the correlation values obtained between sedimentation value, protein content and loaf volume, in addition to that, it varied according to the different quality classes, locations and genotypes.

In relation to quality classes, the highest coefficient of correlation was assigned to the samples from 2013, belonging to wheat B-class and obtained with HMW concentration, while samples of E-class and A-class didn't show any significant correlation. This outcome demonstrated that quality class doesn't affect significantly the relationship between GMP fractions and loaf volume of soft wheat flour samples. Flour samples from the harvest year 2013, displayed a positive significant correlation between GMP, HMW, LMW concentrations over all locations (except location 2), and a negative correlation between loaf volume and LMW/HMW ratio.This outcome is in accordance with the conclusion of Wieser and Zimmermann (2000), Jin et al. (2011), Dhaka and Khatkar (2014) who suggested that HMW plays a positive role in dough rheological properties by contributing more to dough strength than the LMW, and illustrated the dependence of baking quality on LMW/HMW specifying that higher HMW concentration in comparison with LMW concentration lead to a higher baking quality. Moreover, among locations and genotypes the highest correlation achieved with loaf volume fluctuates from GMP, HMW, LMW to LMW/HMW ratio. Those findings indicate that the relationship between loaf volume and GMP subunits concentrations significantly changed with location and genotype, confirming previous study (Zhu and Khan, 2001), which supposed that environment and genotype affected the formation of GMP by modifying molecular sizes of glutenin polymers by influencing the rate of protein synthesis and incorporation of HMW and LMW subunits. Nevertheless, almost no location and genotype in 2014 represents a significant correlation between loaf volume and GMP subunits, which might be explained by the effect of heat on the genotypes present in these locations, which disturbed the biosynthesis of GMP subunits and subsequently gave a non-significant estimation of the relationship between them and loaf volume, as well as by the effect of high

kneading during the baking test, which can lead to a disaggregation of the GMP-Network, generating no significant correlation between loaf volume and GMP fractions.
Moreover, the nature of wheat varieties has a pivotal influence on the assessment of the quality characteristics, because they differed in degree of responsiveness to the environment. So that, one or two samples belonging to a set of samples taken for a study, that are very responsive to the environmental changes, or the other way around, could mainly reduce the correlation values obtained and lead to incorrect conclusions regarding the environment influence on the quality characteristics (Lukov & McVetty, 1991).
GMP, HMW and LMW concentration are not the only factors affecting the relationship with loaf volume, the composition of HMW impacts also the GMP concentration and the loaf volume (Shewry et al., 1992), which needs to be investigated on the basis of a multigenotype and multilocation analysis.

Besides the assessment of wheat baking quality of pure wheat flour samples (previously investigated), the prediction of wheat blend flour samples was also a question of interest of the current study. However, by blend flour samples, a synergetic effect is observed when the loaf volume of flour blend exceeds or is inferior to the loaf volume mathematically assumed from the loaf volumes of the wheat flours blended. This effect is known as blending effect. Therefore, before the assessment of blend baking quality and the prediction of loaf volume, the assessment of blending effect value (BEV) was carried out taking into account the impact of genotype, location of growth and another essential factor which is the level of nitrogen fertilisation. A major advantage might be benefited from the blending effect, which is an improvement of the blend loaf volume while reducing nitrogen fertilisation for economic and ecological aims.
A few of the available studies enquired into the clarification of the causes of the blending effect and its influences on the dough properties and the baking performances. Bushuk et al. (1983) supposed that this effect could be a result of synergistic effects of the different protein qualities of the wheat flours blended and doesn't depend on their protein content. While Bolling (1980; 1983) reported that the blending effect and the rheological dough properties are closely related to each other, indicating that complementarity between wheat varieties is achieved. Thus, Schäfer and Flechsig (1985) demonstrated that wheat varieties with soft dough properties are strengthened by the means of wheat varieties with strong dough properties, dealing to an increase of the loaf volume of the wheat blends, and the more different the stretch resistance and elasticity dough properties of the wheat flours blended is

the greater blending effect is obtained. As a result, the relationship between the aggregation gluten properties through GPT, the rheological dough properties by means of the Extensograph, the GMP concentrations, the blending effect value and obviously loaf volume of wheat flour blends was investigated, highlighting the effect of genotype, location and level of fertilisation (low, medium, high).

As reported in Chapter 5, the BEV which corresponds to the difference between the observed loaf volume of the blend obtained from the RMT baking test and the calculated loaf volume obtained from the loaf volume values of the pure flour samples integrated in this blend, can be positive, negative or insignificant (BEV less than ± 15 ml/100g of flour). According to the results obtained in the present study, it seems that location, genotype, level of fertilisation and their interaction have a large influence on the BEV. However, it seems that the high level of late nitrogen fertilisation doesn't have an impact on the BEV, which achieves the highest values at medium fertilisation level. This observation appears to be confirmed by the stability of the BEV of the blend Memory – Julius over the medium and high fertilisation levels in location 1 (42 ml / 100g and 41 ml / 100g, respectively), nevertheless this stability might be also the results of the complementarity between the blending partners Memory and Julius, which are the varieties with the most complementary dough properties, as demonstrated by the Extensograph data (data not shown). Nevertheless, the ANOVA analysis (Chapter 5, Table 2) confirmed that a high amount of nitrogen fertilisation doesn't apply automatically a positive and high BEV, because medium fertilisation level can lead to the same blending effect achieved with high level. This BEV appears to be not influenced by the interactions genotype × fertilisation level and genotype × location but turned out to be conditioned by the interaction location × fertilisation level, which means the magnitude of the significant difference between the different fertilisation levels depends on which location; the blending partners composing the blends are from. But this interaction was only observed in some locations between low and high level and between low and medium fertilisation level, while regarding medium vs. high, any significant difference was obtained over the all locations. These results are confirming our previous observation and approve that a reduced nitrogen fertilisation from high to medium level doesn't impact the BEV and the same result can be achieved either with medium or high fertilisation level. Our outcome cannot be compared with other studies, because the effect of genotype, location and fertilisation level on blending effect has not been previously investigated and needs to be further elucidated.

GPT parameters, GMP and GMP subunits concentrations and Extensograph parameters, which displayed a wide range of variation by genotypes, locations and fertilisation levels,

were investigated to assess the BEV and the loaf volume of wheat flour blends. In this purpose, and since the prediction of blending effect has not been previously investigated, there is not yet a specific method to predict the BEV and the loaf volume of blends, two methods were applied. These methods are based on the quality characteristics of the blending partners, that means GPT parameters, GMP subunits concentrations and Extensograph parameters of the two pure wheat flour samples involved to make each blend. The first method is based on the mean values of the parameters considered of the two flour samples forming the blend (method 1:$\bar{X}$), while the second method involves the absolute difference between the two flour samples results forming the blend (method 2: Δ). The second method was taken because of the study of Bolling (1968), who suggested that the greater the difference between the rheological dough properties of the flour samples forming the blend, the greater blending effect value is obtained. The outcome of the correlation conducted between the BEV and the parameters investigated demonstrated that the highest and significant correlation was obtained between BEV and ΔHMW ($r = 0.65$, $p < 0.001$), (also with $GMP_{\bar{X}}$: $r = 0.59$, $p < 0.001$) and was only achieved at high fertilisation level. These correlations increased from low to high fertilisation level (except $HMW_{\bar{X}}$ and ΔGMP at medium level), which indicates that the fertilisation level plays an important role in the ability of the GMP subunits concentrations to predict BEV. That can be explained by the large impact of nitrogen fertilisation on the GMP and HMW concentrations, so that nitrogen enhances HMW synthesis and improves HMW concentration, which is closely correlated with GMP concentration as reported by Yue et al. (2007). Whereas, the relationship between Extensograph parameters, calculated according to the method 2, and BEV remained low, which didn't confirm the outcome of Bolling (1984b), who suggested that a high difference between the extensibility values of the blending partners leads to a high blending effect. In addition to that the analyse of the variation of BEV of all blends showing a positive blending effect according to the ΔE values (data not shown) didn't fit with this suggestion to. The difference between our outcome and the results of this study may be due to the presence of just 18 blend flour samples that have a positive blending effect value among 135 blend flour samples. Moreover Bolling (1968) didn't investigate blend flour samples with negative BEV, which is the case in our study.

In contrast, among the parameters investigated, the Extensograph parameter $R_{\bar{X}}$ (45min) (resistance to extension measured after 45 min proofing time) calculated according to method 1 showed the highest and significant correlation with loaf volume of all blends ($r = 0.66$, $p < 0.001$), while GPT parameters and didn't display a significant relation to loaf volume. This

relationship remained also significant over all fertilisation levels (r = 0.67, p < 0.001 at low level, r = 0.75, p < 0.001at medium level and r = 0.74, p < 0.001 at high level), but it was more stable against nitrogen fertilisation increase from medium to high level, than from low to medium level. The Extensograph parameter $(R/E)_{\bar{X}}$ ratio at 45 min proving time displayed also a good correlation with loaf volume over the three level of fertilisation (r = 0.57, p < 0.001 at low level, r = 0.73, p < 0.001at medium level and r = 0.68, p < 0.001 at high level). These results are in accordance with the outcome of Ktenioudaki et al. (2011), who demonstrated a significant relationship between loaf volume and dough rheological parameters. $GMP_{\bar{X}}$ and $LMW_{\bar{X}}$ at low level showed also a significant correlation to loaf volume (r = 0.62, p < 0.001 and r = 0.64, p < 0.001, respectively), however this relationship became not significant with the increase of nitrogen fertilisation level, which is in contrast with the results of Yue et al. (2007). That might be due to the impact of the environmental factors, such as location, genotype, and their interaction with the nitrogen fertilisation level as reported by Triboi et al. (2000), which exceeds the effect of the increase of fertilisation level on GMP. Moreover, particle size of the GMP subunits is also an important factor in determining wheat baking quality, and the effect of fertilisation level on it also can influence our outcome.

Since the relationship between the BEV and the parameters investigated remained non-significant, a best subset regression analysis was performed with BEV of all blends from the three fertilisation levels as dependant variable and protein content, sedimentation value, ΔGMP subunits concentrations and all Δ Extensograph parameters from the three proving times were included in the analysis as independent variables, in order to develop a model enabling a better prediction of BEV. However, all models produced didn't provide a significant explanation of the variation of the BEV. That means the Extensograph parameters (En, R, E, R/E ratio) even in combination with GMP and GMP subunits concentrations (GMP, HMW, LMW, LMW/HMW ratio), protein content and sedimentation value are not good predictors of BEV of blend flour samples from different fertilisation levels. The relationship between these parameters and BEV remains dependent on the level of fertilisation, genotype, location and their interactions.

But on the other hand, this Extensograph parameter $R_{\bar{X}}$ (45 min) in combination with $GMP_{\bar{X}}$ protein content and sedimentation value turned out to be good predictors of loaf volume of blend flour samples (R^2 = 0.69), That means the model created by means of the mentioned parameters can provide an explanation of loaf volume variation by 69% with an uncertainty of ± 23 ml, which is better than the uncertainties of the other models (chapter 5, Table 4).

Afterwards a cross validation was performed while partitioning our data according to each fertilisation level in order to test if the results obtained can be more generalizable and that the model will fit other samples. This resulted in good correlation between observed and predicted loaf volume over the three fertilisation levels ($r = 0.89$, $p < 0.001$ at low level; $r = 0.84$, $p < 0.001$ at medium level and $r = 0.83$, $p < 0.001$ at high level) (Chapter 5, Figure 4). Those results point out that the model created involving the mean values of the blend partners of the followed parameters: protein content, sedimentation value, GMP concentration and the Extensograph parameter R (45 min), can be useful to explain variation in loaf volume of blends whichever is the level of nitrogen fertilisation (low, medium or high), and subsequently predict the BEV that is normally noticed only by means of the baking tests.

2. General conclusions

The assessment of baking quality of soft wheat flour samples through the prediction of loaf volume by means of new methods is nowadays of major importance, since the baking tests require specific equipment of baking facilities, experienced staff, time, and a considerable quantity of wheat material, so that they are not suited to be performed with a limited amount of wheat flour and under a time constriction. Moreover, the classical quality parameters appear to be no longer suitable to assess the baking quality of modern wheat varieties. Thereby, it is in this context that the present thesis has been carried out.

The current work is structured in three main parts; firstly the prediction of bread wheat baking quality using an optimized GlutoPeak®-Test method (GPT), followed by the analysis of the relationship between glutenin macro polymer (GMP) and bread wheat baking quality on the basis of multilocation and multigenotype analysis, and finally the prediction of blending effect and baking quality of soft wheat blends by means of Extensograph, GPT and GMP under variable fertilisation levels and environmental conditions.

To deal with the first work package, 15 different bread wheat varieties from the harvest year 2013, and 11 varieties from the harvest year 2014, cultivated at eight different locations in Germany ($n = 120$, 2013; $n = 88$, 2014) were analysed by means of the GPT after optimisation of the GPT method, using different solvents (distilled water, 5 % sucrose and 5 % sodium carbonate, 5 % lactic acid and 1 % sodium chloride) and varying the flour/solvent ratio. Correlations were established between crude protein content, sedimentation value, the gluten aggregation behaviour parameters measured by means of GPT and loaf volume. Finally, a multiple regression analysis was accomplished to highlight the relationship between

loaf volume and various screening tests. The best fit model was validated by an independent set of data from the harvest year 2015.
The results of this part showed that among the solvents used to run the GPT the 1 % sodium chloride solution was the most convenient one to obtain a significant and environmental stable response regarding the loaf volume. Analysis exhibits that the influence of years on the stability of the method is of more importance than locations. A linear function of protein content and the GPT parameter AM was found to be useful to explain the variation in loaf volume over different location and year combinations. The evaluation of this linear function with an independent data set leads to 64 % correct and to 36 % incorrect predictions. This indicates that the strategy of combining two rapid tests is effective to a certain degree (e.g. for variety screening) and that the application of the model can be a useful approach in breeding for improved baking quality varieties in bread wheat, but it cannot be expected to replace the actual measurement of loaf volume via standard baking test.

In the second work package, the GMP of 15 different soft winter wheat varieties from the harvest year 2013 and 11 different soft winter wheat varieties from the harvest year 2014, cultivated at eight different locations in Germany were extracted under reducing conditions and quantified using HPLC. The impact of genotype and location of growth on loaf volume, the Correlations between GMP subunits concentrations; (GMP, high molecular weight (HMW), low molecular weight (LMW)), LMW/HMW ratio and loaf volume were demonstrated as well as the influences of variety and location on the GMP concentration.
The results obtained demonstrated that the GMP subunits concentrations of wheat flour samples from the harvest year 2013 were more influenced by genetic than environmental factors, in contrast with GMP subunits concentrations of wheat flour samples from the harvest year 2014, emphasising the major effect of environment and genotype on the GMP fractions. The relationship between loaf volume and GMP subunits concentrations of wheat flour samples from 2013 comes out in some cases significant and in others non-significant because it remains under the influence of the genetic background of the samples and the location of growth. This relationship is revealed non-significant for wheat flour samples from 2014, due to the heat effect, characterising this year, and which can lead to a decrease in GMP. This indicates that the GMP subunits concentrations cannot be a useful approach to replace the baking test and predict the loaf volume without considering the effect of the environment and genotype. An investigation of the composition of the GMP subunits might be also important to highlight the process involved in this relationship with loaf volume.

Finally, in the third work package six different soft wheat varieties from the harvest year 2015 cultivated at three different locations in Germany and with three different levels of nitrogen fertilisation (low, medium and high level of fertilisation) as well their flour blends samples resulting from a blending to a ratio of 50:50 were tested in this study. The gluten aggregation properties of the pure and the blend flour samples were investigated by means of GPT, the rheological dough properties by means of the Extensograph, while the GMP were extracted under reducing conditions and quantified by HPLC.

The outcome reported that the blending effect which can be positive, negative or insignificant is influenced by genotype, location, fertilisation level and the interaction location × fertilisation level. However no significant difference was obtained between high and medium fertilisation level over the locations considered, thus implying that the late season fertilisation with a high level doesn't affect the BEV and the reduction of nitrogen fertilisation from high to medium level is possible. However, fertilisation level remains a crucial source of variation of the relationship between GPT, GMP and Extensograph parameters calculated on the basis of the quality characteristics of the blending wheat flour partners, and the BEV, which makes its prediction by means of those parameters even in combination not possible. Among these parameters, the resistance to extension (R) at 45min showed the highest, significant and environmental stable correlation with blends loaf volume. A linear function of R (45 min), GMP, protein content and sedimentation value can provide an explanation of blends loaf volume variation by 69% with an uncertainty of ± 23 ml, whichever is the level of nitrogen fertilisation. Further studies are necessary to strengthen these results and investigate the effect of different blending ratios on the BEV, on the basis of a multiyear, -genotype and –location analysis in the purpose to take advantage of the blending effect , considered as a new approach to increase the baking quality of soft wheat varieties and to reduce nitrogen fertilisation level.

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Abstract:

The overall objective of the present thesis was to assess the baking quality of soft wheat flour samples (*Triticum aestivum* L.), through the estimation of the loaf volume by means of new methods, since the baking tests are not suited to be performed with a limited amount of wheat material and under a time constriction, and the crude protein content appear to be no longer appropriate to assess the baking quality of modern German wheat varieties. The methods applied are the GlutoPeak®-Test (GPT) as a rapid small-scale technique to evaluate the gluten aggregation properties, and the gluten macro polymer (GMP) subunits concentrations, extracted under reducing conditions and quantified by means of the HPLC. The effect of genotype and environmental conditions on the relationship between loaf volume and the quality parameters measured was emphasized through multilocation and multigenotype analysis. In addition to these methods, the rheological dough properties of soft wheat flour blends were also determined by means of the Extensograph, in order to assess the blending effect value and loaf volume of flour blends, highlighting the impact of genotype, environmental conditions including location of growth and fertilisation level.

The more significant and stable GPT response was achieved with 1 % sodium chloride, as a solvent to run the GPT. The best prediction of loaf volume a linear function of protein content and the torque 15 s before maximum torque (AM), explaining the variation in loaf volume by 63 % and providing an uncertainty of ±39 ml. The accuracy of the validation of the linear function leads to 64 % correct and to 36 % incorrect predictions of the loaf volume. This emphasizes that the application of the linear function of protein content and AM cannot replace the actual measurement of loaf volume, but it could be a useful rapid screening test in breeding for improved baking quality in bread wheat.

The relationship between loaf volume and GMP subunits concentrations changed significantly according to genotype and location of growth for wheat flour samples from the harvest year 2013, however wheat flour samples from 2014 didn’t represent a significant correlation between loaf volume and GMP subunits concentrations, due to the heat effect, characterising this year, and which can lead to a decrease in GMP. Furthermore, this relationship does not reach the correlation values obtained between sedimentation value, protein content and loaf volume. This highlights that GMP subunits concentrations cannot predict the loaf volume without taking into account the effect of genotype and location, besides the composition of high molecular weight which also impacts the GMP concentration and the loaf volume.

Genotype, location, fertilisation level and the interaction location × fertilisation level affected also significantly the blending effect value (BEV), the synergistic effect noticeable by blend flour samples; however, the transition from medium to high fertilisation level over all locations didn't have any effect. A linear function of the mean value of the resistance to extension (R) values of the blending flour samples partners measured at 45 min, GMP concentration, protein content and sedimentation value can provide an explanation of loaf volume variation by 69% with an uncertainty of ± 23 ml. The evaluation of this model resulted in good correlation between observed and predicted loaf volume over the three fertilisation levels ($r = 0.89$, $p < 0.001$ at low level; $r = 0.84$, $p < 0.001$ at medium level and $r = 0.83$, $p < 0.001$ at high level), which emphasises that this model could be useful to explain variation in loaf volume of blends whichever is the level of fertilisation, and subsequently predict the BEV that is normally noticed only by means of the baking tests.

Taken together, the results obtained in this thesis provide new insights into:

- The AM GlutoPeak®-Test parameter in combination with crude protein content cannot replace the actual measurement of loaf volume, but it could be a useful rapid screening test in breeding for improved baking quality in bread wheat.
- Genotype, location of growth and the composition of HMW have an important effect on the ability of GMP subunits concentrations to predict the loaf volume.
- The mean values of the blending flour samples partners of R (45 min) Extensograph parameter, in combination with GMP concentration, crude protein content and sedimentation value can predict the loaf volume of soft wheat blends, whichever the level of nitrogen fertilisation. Therefore, decreasing the level of late nitrogen fertilisation seems to be possible in terms of preparation of wheat blends.

Keywords: Loaf volume, Protein content, Sedimenttaion value, GlutoPeak®-Test, Gluten macro polymer, Location, Genotype, Blending effect, Nitrogen fertilisation level.

Résumé :

L'objectif global de la présente thèse était d'évaluer la qualité boulangère des échantillons de farine de blé tendre (Triticum aestivum L.), par l'estimation du volume du pain grâce à de nouvelles méthodes, puisque les tests de panifications ne sont pas adaptés pour être effectués avec une quantité limitée de farine de blé et sous une contrainte de temps, et la teneur en protéine brute ne semble plus appropriée pour évaluer la qualité boulangère des variétés de blé allemandes modernes. Les méthodes appliquées sont le GlutoPeak®-Test (GPT) en tant que technique rapide pour évaluer les propriétés d'agrégation du gluten et les concentrations en macro-polymère de gluten (GMP) et de ses sous-unités extraites dans des conditions réductrices et quantifiées par HPLC. L'effet du génotype et des conditions environnementales sur la relation entre le volume du pain et les paramètres de qualité mesurés a été souligné par l'analyse multi-environnemental et -génotypique. En plus de ces méthodes, les propriétés rhéologiques de la farine de blé tendre ont également été déterminées à l'aide de l'Extensographe, afin d'évaluer la valeur de l'effet de mélange (BEV) et le volume du pain, mettant en évidence l'impact du génotype, les conditions environnementales, et le niveau de fertilisation azotée.

La réponse du GPT la plus significative et la plus stable a été obtenue avec 1% de chlorure de sodium. La meilleure prédiction du volume du pain est une fonction linéaire de la teneur en protéines et du couple 15 s avant le couple maximum (AM), expliquant la variation du volume du pain de 63% et fournissant une incertitude de ± 39 ml. La validation de la fonction linéaire conduit à 64% de prédictions correctes et à 36% incorrectes du volume du pain. Cela souligne que l'application de la fonction linéaire de la teneur en protéines et AM ne peut pas remplacer la mesure réelle du volume du pain, mais il pourrait être un test de dépistage rapide utile dans la sélection pour améliorer la qualité boulangère du blé panifiable.

La relation entre le volume du pain et les concentrations en GMP et ses sous-unités change significativement selon le génotype et le lieu de croissance des échantillons de farine de blé de l'année de récolte 2013, cependant les échantillons de farine de blé de l'année 2014 ne montrent aucune corrélation significative à cause de l'effet de chaleur, caractérisant cette année, et qui peut conduire à une diminution de la formation du GMP. De plus, cette relation n'atteint pas les valeurs de corrélation obtenues entre la valeur de sédimentation, la teneur en protéines et le volume du pain. Ceci met en évidence que les concentrations en GMP et ses sous-unités ne peuvent pas prédire le volume du pain sans prendre en compte l'effet du génotype et du lieu de croissance, outre la composition des sous-unités du GMP qui influence également la concentration en GMP et le volume du pain.

Le génotype, le lieu de croissance, le niveau de fertilisation et l'interaction lieu de croissance × niveau de fertilisation ont également affecté significativement la BEV. Cependant, à travers les différents lieux de croissance, le passage d'un niveau de fertilisation moyen à élevé n'a eu aucun effet. Une fonction linéaire de la valeur moyenne des échantillons partenaires du mélange de résistance à l'extension (R à 45 min), concentration en GMP, teneur en protéines et valeur de sédimentation peut fournir une explication de la variation du volume du pain de 69%, avec une incertitude de ± 23 ml. L'évaluation de ce modèle a conduit à une bonne corrélation entre le volume de pain observé et prédit sur les trois niveaux de fertilisation ($r = 0,89$, $p < 0,001$ au niveau faible, $r = 0,84$, $p < 0,001$ au niveau moyen et $r = 0,83$, $p < 0,001$ un niveau élevé), qui souligne que ce modèle pourrait être utile pour expliquer la variation du volume du pain des mélanges, quel que soit le niveau de fertilisation, et prédire par la suite la BEV qui n'est normalement observé qu'au moyen des tests de panification.

Pris ensemble, les résultats obtenus dans cette thèse fournissent de nouvelles perspectives sur:

- Le paramètre AM du GlutoPeak®-Test associé à la teneur en protéines brutes ne peut pas remplacer la mesure réelle du volume du pain, mais il pourrait constituer un test de dépistage rapide utile en phase de sélection pour améliorer la qualité boulangère du blé tendre.
- Le génotype, le lieu de croissance et la composition de HMW ont un effet important sur la capacité des concentrations en GMP et ses sous-unités à prédire le volume du pain.
- Les valeurs moyennes des échantillons partenaires intégrés dans les mélanges de farine du paramètre d'extensographe R (45 min), en association avec la concentration en GMP, la teneur en protéines brutes et la valeur de sédimentation peuvent prédire le volume de pain de blé tendre, quel que soit le niveau de fertilisation azotée. Par conséquent, la réduction du niveau de la fertilisation azotée tardive semble possible en termes de mélanges de blé.

Mots-clés : Volume du pain, Teneur en protéines, Valeur de sédimentation, GlutoPeak®-Test, Macro-polymère du gluten, Lieu de croissance, Génotype, Effet de mélange, Niveau de fertilisation azotée.