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PhD Thesis

High amylose wheat flours for the development of healthy cereal based foods

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SUMMARY

According to scientific evidence, the diet has a central role in the promotion of health, and foods are now perceived as tools intended not to only satisfy physiological requirements but also to prevent nutrition-related diseases. In this context, food technologists are working on the development of innovative and healthy foods also to meet the growing consumer demand for this type of products. Based on this, cereals play an important role, because they are a common source of carbohydrates, protein, dietary fibre, vitamins and minerals, but they can also carry many bioactive compounds.

Among cereals, wheat is a versatile ingredient for the production of foods with a high sensory appeal and popularity, indeed, wheat-based foods are ubiquitous in Western diets.

However, nutritional quality of refined wheat foods is lower than the corresponding wholegrain products, due to the removal, during milling, of the caryopsis peripheral layers and germ, in which minerals, vitamins and especially dietary fibre are concentrated.

To improve the nutritional quality of refined wheat-based food, manipulation of starchy endosperm targeting for changing the amylose/amylopectin ratio in favor of amylose could provide substantial health benefits at a global level through the delivery of resistant starch.

Resistant starch is the starch fraction that escapes digestion and absorption in the upper gut and consequently reaches the large bowel where it serves as a substrate for the colonic microbiota. This property is able to provide fundamental support both to gut cell and other extra-intestinal tissues.

In light of these considerations, this research aims to produce innovative cereal products with enhanced nutritional and healthy properties using high amylose wheat flours.

The research work includes four main activities:

1) physicochemical and nutritional characterization of grain samples: the wild-type wheat line Cadenza (*Triticum aestivum* L.) and the modified high amylose line;
2) development of milling diagrams using both soft and hard wheat mill in combination with different debranning and tempering condition in order to obtain a higher amount of refining flours;
3) use of the obtained high amylose flours, alone and in combination with conventional ones, for the production of pasta;
4) nutritional, healthy and cooking properties evaluation of experimental pasta samples.

The results obtained in the first part of the work highlighted that the modification of amylose-amylopectin ratio causes chemical (e.g. increase of resistant starch) and physical (e.g. increase of grain hardness) changes in kernel characteristics.

A further important result of this study was the obtaining of flours with different chemical and rheological characteristics that allow their employment in the production of a wide range of cereal products with enhanced nutritional and healthy properties thanks to higher content of fibre and resistant starch, in compliance with nutritional and healthy *claims* enlisted in EC Reg. 1924/2006 and EU Reg. 432/2012.

In particular, in this work the semolina-like flour obtained from durum wheat milling diagram was used, in combination with durum wheat semolina, for the production of pasta samples.

On the basis of percentage substitution of durum wheat semolina with high amylose semolina, pasta samples presented levels of fibre and resistant starch that fulfill the requirements of nutritional and healthy *claims* above mentioned.

RIASSUNTO

Numerose evidenze scientifiche hanno dimostrato che la dieta ha un ruolo centrale nella prevenzione delle malattie, infatti gli alimenti non solo hanno il compito di soddisfare i fabbisogni fisiologici dell'organismo, ma possono anche prevenire malattie legate all'alimentazione. In questo contesto, i tecnologici alimentari lavorano per lo sviluppo di alimenti innovativi e salutistici anche per rispondere alla crescente richiesta da parte dei consumatori di questa tipologia di prodotti.

Sulla base di queste considerazioni, i cereali giocano un ruolo importante, poichè sono una fonte di carboidrati, proteine, fibra alimentare, vitamine e minerali, inoltre contengono anche diversi composti bioattivi.

Tra i cereali, il frumento è un ingrediente versatile e molto diffuso e i prodotti da esso derivati sono ubiquitari nella dieta dei Paesi occidentali.

Tuttavia, i prodotti finiti a base di sfarinati di frumento raffinati presentano una qualità nutrizionale inferiore rispetto ai corrispettivi ottenuti da sfarinati di frumento integrale a causa dell'allontanamento, durante la macinazione, degli strati esterni della cariosside e del germe che sono ricchi di minerali, vitamine e soprattutto fibra alimentare.

Per migliorare la qualità nutrizionale dei prodotti finiti, le modifiche genetiche che inducono nel frumento un incremento del rapporto amilosio/amilopectina potrebbero fornire benefici salutistici legati all'apporto di amido resistente.

Quest'ultimo è definito come la porzione di amido che non viene digerita nell'intestino tenue e raggiunge il colon dove viene fermentato dai microrganismi presenti. Questa proprietà è capace di apportare effetti benefici sia alle cellule dell'intestino che a quelle dei tessuti extra-intestinali.

Questo progetto di ricerca ha lo scopo di produrre alimenti innovativi a base di cereali con proprietà nutrizionali e salutistiche migliorate usando sfarinati di frumento ad alto contenuto di amilosio.

Il presente lavoro di tesi include quattro attività principali:

1) caratterizzazione chimico-fisico e nutrizionale di campioni di grano della varietà Cadenza ad alto contenuto di amilosio (*Triticum aestivum* L.) e della corrispondente varietà controllo;
2) sviluppo di diagrammi di macinazione usando sia mulini a tenero che a duro in combinazione con differenti condizioni di decorticazione e condizionamento del grano di partenza al fine di ottenere sfarinati raffinati;
3) uso degli sfarinati ottenuti, tal quale o in combinazione con sfarinati convenzionali, per la produzione di pasta;
4) valutazione delle proprietà nutrizionali e salutistiche e della qualità di cottura della pasta ottenuta.

I risultati ottenuti nella prima parte del lavoro hanno evidenziato che la modifica del rapporto amilosio/amilopectina induce cambiamenti nelle caratteristiche chimico-fisiche della cariosside, in particolare si riscontra un incremento dell'amido resistente e della durezza della cariosside.

Un altro importante risultato di questo studio è stato l'ottenimento di tipologie di sfarinati con caratteristiche chimiche e reologiche differenti che ne permettono l'impiego nella produzione di una vasta gamma di prodotti a base di cereali con migliorate caratteristiche nutrizionali e salutistiche grazie all'alto contenuto di fibre e di amido resistente, in accordo con i *claim* nutrizionali e salutistici riportati nei Regolamenti Comunitari CE 1924/2006 e UE 432/2012.

In particolare, in questo lavoro, la semola alto amilosio ottenuta dalla macinazione con mulino a duro è stata utilizzata, in combinazione con semola di grano duro, per la produzione di pasta.

Sulla base della percentuale di sostituzione di semola di grano duro con la semola alto amilosio, i campioni di pasta presentavano livelli di fibra alimentare e amido resistente che soddisfacevano i requisiti dei *claim* nutrizionali e salutistici menzionati.

GENERAL INTRODUCTION

Diet-related chronic diseases, such as coronary heart disease and diabetes, are major causes of morbidity and mortality in both affluent industrialized countries and developing countries. Increased consumption of wholegrain cereal foods is recognized as an important approach for reducing the risk of these prevalent health problems. The benefits of whole grains are largely attributed to their dietary fiber component, which includes non-starch polysaccharides and resistant starch (RS) (Belobrajdic et al., 2019).

Although people are eating more whole grains, refined starches from cereals, which elicit a high glycemic response, continue to be the main form of carbohydrates consumed across the globe. For this reason, the attention of food scientists is focused on the selection of appropriate dietary fibre to produce healthier foods with acceptable quality attributes.

The use of resistant starch, rather than bran fraction, to improve food nutritional quality has been tested extensively for the production of cereal products such as bread and pasta made replacing part of the wheat flour with resistant starch extracted from natural high amylose starches (Homayouni et al., 2014). However, the examination of the end-use of high amylose wheat for the production of innovative cereal food naturally rich in resistant has not been studied plentifully.

Technological features of high amylose wheat

In bread wheat (*Triticum aestivum* L.) seeds, the typical ratio between amylopectin and amylose is 3:1. To increase this ratio in the endosperm, research has primarily focused on abolishing two enzymes essential to amylopectin synthesis, starch synthase

IIa (SSIIa), which is responsible for elongation of amylopectin chain, and starch branching enzyme II (SBEII), by combining mutant homeologous alleles that code for the enzymes (Hogg & Giroux, 2019). The impact of these two types of mechanisms on increasing level of amylose content, on structure and morphology of starch granules and on grain composition is widely different.

Once high amylose wheat lines were obtained, the evaluation of technological performance for the production of cereal products is necessary and fundamental.

Since the breeding was primarily aimed at changing starch composition, it was assumed that the differences in the technological properties would be reflected mainly in the change of the viscous properties.

When investigating the pasting behaviour of a slurry matrix with the RVA (Rapid Visco Analyzer) method, high-amylose wheat lines present a very low viscosity, and viscous properties do not really change during analysis. The baking properties of high-amylose wheat are not as favorable in term of crumb softness during bread storage and bread volume, but their viscous properties can improve the quality of noodles/pasta. Due to their improved gel-forming properties they can be used as thickeners. They also have a beneficial effect on health due to their higher content of resistant starch (Jaksics et al., 2020).

Newberry et al. (2018) produced breads with refined high amylose flour at incorporation levels of 60, 80, and 100% of total flour content. All levels produced doughs with good handling and normal consistency properties; dough extensibility during shaping increased with high amylose flour incorporation level. The use of white high amylose flour in bread formulations allowed for the production of white bread with a high fiber content, without the use of modified ingredients.

Regarding pasta production, Sissons et al. (2020) used high amylose semolina from durum wheat with a high amylose content for the production of spaghetti. The amount of resistant starch and total dietary fiber was significantly higher compared to semolina spaghetti.

High amylose spaghetti had a very similar appearance compared to the control pasta, in particular high amylose pasta was a little duller (lower L*), with more redness and was less yellow than semolina pasta. In contrast, a commercial wholemeal pasta appeared much darker, red and more grainy. Clearly the high amylose pasta had a much more desirable appearance than the commercial wholemeal pasta. In addition, high amylose pasta showed slightly reduced firmness and increased cooking loss. The authors concluded that the innovative pasta highlighted an acceptable quality.

Depending on the type of resistant starch in these food products, the use of various food additives may be necessary to produce foods with acceptable quality attributes. The development of food products with increased resistant starch contents and good quality attributes is imperative to provide foods with increased health benefits and a high acceptability by the consumers (Birt et al., 2013).

Nutritional and healthy properties of resistant starch

The mechanisms involved in enzyme resistance of resistant starch are 1) starch granules maintained intact due to intertwining of amylose among the amylopectin crystallites, 2) restricted swelling of starch granules during heating due to increased amylose-lipid complexes and 3) retrogradation of amylose and very long amylopectin branches to highly ordered crystalline structures on storage of gelatinized starch (Bird & Regina, 2018).

By definition, resistant starch escapes digestion in the small intestine and, accordingly, does not contribute directly to postprandial rises in blood glucose and insulin concentrations, unlike rapidly digested starches. To rank the products with respect to their influence on postprandial glycemia is used glycemic index. For this important characteristic, the intake increase of low glycemic index (GI) foods is recommended in diabetic subjects and people with impaired glucose tolerance, but resistant starch in diet can help to maintain a good health also in non-diabetic person.

Foods with increased resistant starch provide improved digestive system health stimulating the growth and/or the activity of one or a limited number of microorganisms already present in the colon acting as a prebiotic. In particular resistant starch extends the viability of some probiotic microorganisms in the colon. The prolonged fermentation of non-digestible carbohydrates by colon microorganisms in the lower intestine increases production of beneficial short-chain fatty acids by gut microbiota (Hogg & Giroux, 2019), in particular butyrate provides energy to the epithelial cells and inhibits the malignant transformation of such cells. This gives resistant starch also the potential in reducing the development of colon cancer (Raigond et al., 2015) .

The beneficial effects of end-products of fermentation are not only referred to bowel health but also influence metabolism of peripheral tissues, including skeletal muscle, adipose tissue depots and liver.

Substituting conventional (digestible) starches with resistant starches effectively dampens postprandial hyperglycemia and improves glucose control through stimulating insulin sensitivity in peripheral tissues and other interrelated physiological processes, moreover resistant starch contributes to fibre intake and thus promote the sense of satiety, playing an important role in the prevention of overweight and obesity.

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Chapter 1: WHEAT

1.1 Wheat taxonomy

Wheat is the oldest and the most extensively grown grain crops in the world. It is widely accepted that wheat was first grown in the Fertil Crescent, an area which spans present-day Israel, Northern Syria, Southern Turkey, Eastern Iraq, and Western Iran, about 10,000-8,000 B.C. (early neolithic).

In this period there was a wide-scale transition of many human cultures from a lifestyle of hunting and gathering to one of agriculture and settlement.

Many archeological sites dating to Pre-Pottery Neolithic B period (8,800-6,500 B.C.) show evidence of cultivation of domesticated forms of both einkorn and emmer which are ancient species differing from wheat in having the grain enclosed within lemma and palea, thus the grain is "hulled" rather than free-threshing or naked, and a fragile rachis.

Many studies have been conducted to understand the transition from ancient plants to cultivated/domesticated wheat crops. Domestication of wheat resulted from mutations that gave rise to traits such as soft glumes, a non fragile rachis, and the free-threshing character (Simons et al., 2006).

It appears that hulled and free-threshing forms were being cultivated alongside for millennia. Replacement of hulled wheat with free-threshing form was complete over most of Europe by about 950 a.D., but in isolated area, such as southern Germany and northern Switzerland, this did not occur until the twentieth century b.C. (Gooding, 2009).

Wheat, through the millennia, has been intimately associated with human food uses. In the Stone Age, grains were crushed between flat stone; the crushed material was moistened with water and made in a flat cake, which was dried in the sun. The ancient Egyptians perfected the art of baking and then during the classical period of Greek and

Roman domination of Western civilization, baking become an occupation (Wrigley, 2009).

Because of its agronomic adaptability, ease of storage, nutritional goodness and the unique dough-forming property that allows to produce a variety of palatable, interesting and satisfying foods, wheat is the major component in most diets of the world, indeed more than 765 million tonnes (MT) of wheat are grown throughout the world on approximately 216 million hectares of land (FAOStat http://www.fao.org/faostat/en/#data/QCL).

Wheat is mainly utilized as food (67%); however, a significant portion is also used for feed, seed and industrial purposes (20%, 7% and 6%, respectively) (Shevkani et al., 2017). The industrial uses of wheat include its wet milling for the production of starch and gluten (Maningat et al., 2009).

1.2 Wheat morphology and anatomy

Triticum aestivum L. (referred as bread wheat) is the most commonly grown species of wheat, accounting for 90–95% of total production. *T. durum* (durum wheat) is another commonly grown species in global wheat production. The latter wheat is preferred and used primarily for making pasta products (Gélinas et al., 2016), hence, also referred as pasta wheat. Durum wheat has higher levels of proteins and yellow pigments (xanthophylls and carotenoids) than common wheat and also differs from the latter in terms of gluten properties (Janković et al., 2015). Common wheat generally grows well under spring or winters, while durum wheat is well adapted to the hot and dry conditions surrounding the Mediterranean Sea and similar climates in other regions (Shewry & Hey, 2015). Moreover, the popularity of durum wheat is increasing

amongst farmers due to high yield and resistance to rusts and karnal bunt (Kaur et al., 2016).

Other wheat species are only cultivated on small areas, either for cultural reasons or for the expanding market in healthy foods. These are einkorn (diploid *T. monococcum* var. *monococcum*), emmer (tetraploid *T. turgidum* var. *dicoccum*), and spelt (*T. aestivum* var. *spelta*). However, interest in these species has recently increased because of the demand for special breads and beers as well as due to lower requirements of nitrogen fertilizers and crop protection chemicals (Gooding, 2009).

Wheat plants develop through recognizable phases, typical of many annual grasses. The vegetative phase starts with germination, followed by the appearance of leaves and then of 2-3 tillers on each plant. The reproductive stage commences with the development of spike in the flag leaf. The next stage is booting that describes the swelling of the flag leaf and the expands of spike. This is followed by emergence of the spike, flowering (anthesis) and fertilization. Successively the grain fills gradually with dry matter until harvest maturity (Gooding, 2009).

Wheat grains are borne on the spike. The major axis is the rachis that has two rows of spikelets in alternating order. Each spikelet is surrounded with glumes at the base and presents a central axis called rachilla bearing a series of up to six florets. The floret consists of a pair of pales, lemma (the lower) and palea (the upper) enclosing the ovary and, after the fertilization, the caryopsis. Lemma presents a thin appendix, called awn or arista, not always presents (Figure 1.1).

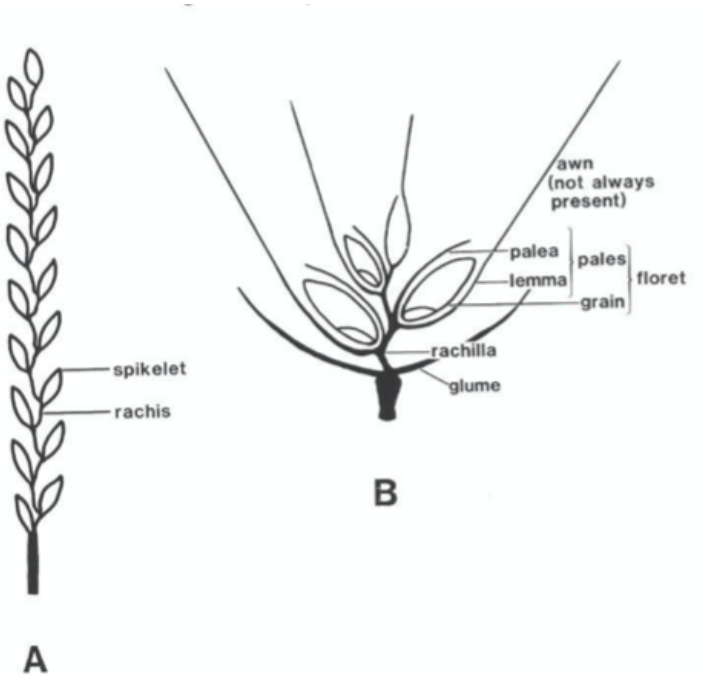

Figure 1.1. A, spikelets arranged alternately on the rachis. B, wheat spikelet components.

The shape of caryopsis is ovoid. The dimension of the kernel varies with the type of wheat and conditions of growth, but the length is generally about 4–8 mm and the width is between 2 and 5 mm. The grain has two distinct sides, one dissected by a crease, known as the “ventral” side, and the other side is rounded and called “dorsal” side. One end of the grain is covered in a fine layer of hairs (brush), the opposite end presents the embryo.

Wheat kernel consists of several layers (Figure 1.2), starting from the outside, it shows:

-peripheral layers (about 5-7% of the caryopsis);

-the aleurone layer (about 8% of the caryopsis);

-starchy endosperm (about 80-85% of the caryopsis);

-germ or embryo (about 3% of the caryopsis).

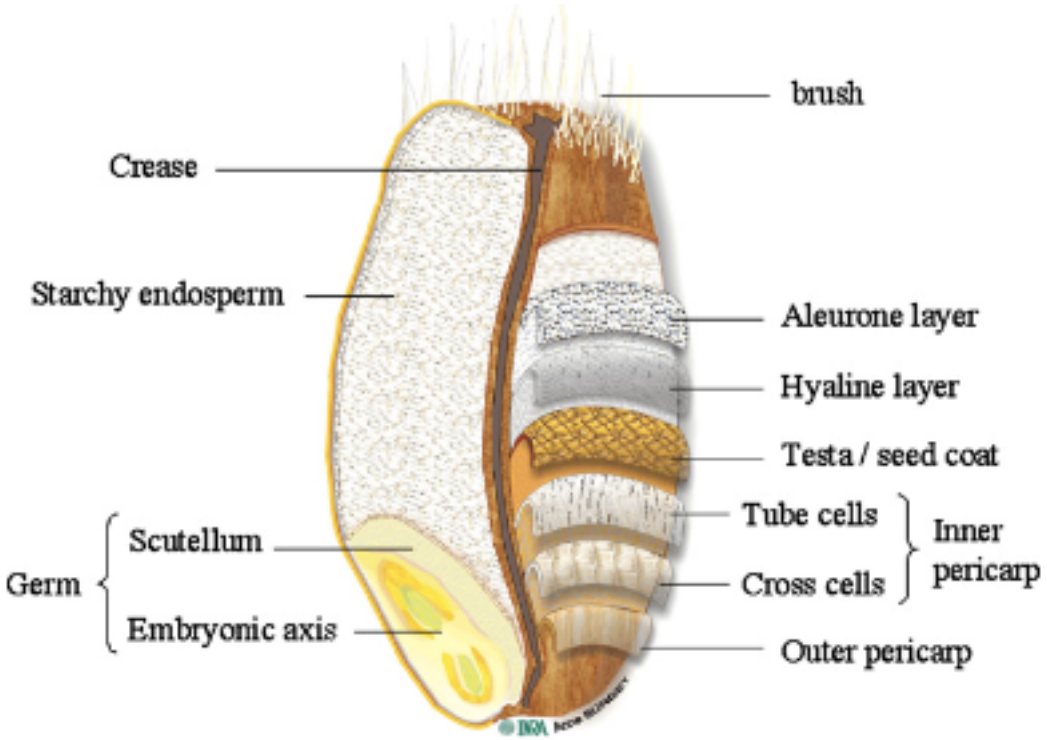

Figure 1.2. Histological composition of wheat grain. From: Hemery et al. (2007).

The peripheral layers, which surround the endosperm, include -in order from the outer to inner parts- the outer and inner pericarp, seed coat and hyaline layer. The peripheral layers of mature grain are multilayered, each tissue presents different thickness and is closely associated with one another. The outer pericarp is the thickest (15–30 μm), instead the seed coat (testa) cells are the thinnest (5–8 μm) (Barron et al., 2007).

The endosperm is composed of aleurone layer and starchy endosperm.

The aleurone layer in wheat is typically only one cell layer thick at maturity (Figure 1.3). It forms the outermost layer of the endosperm tissue, enclosing the starchy endosperm and part of the embryo. The aleurone layer is treated by the miller as part of the bran. The intracellular aleurone content (70% of aleurone dry mass) is characterized by high contents of small vacuoles (aleurone grains), proteins, minerals, phytates, lipids and B vitamin, while the aleurone cell wall is mainly composed of arabinoxylan, β-glucans and proteins (Rosa-Sibakov et al., 2015).

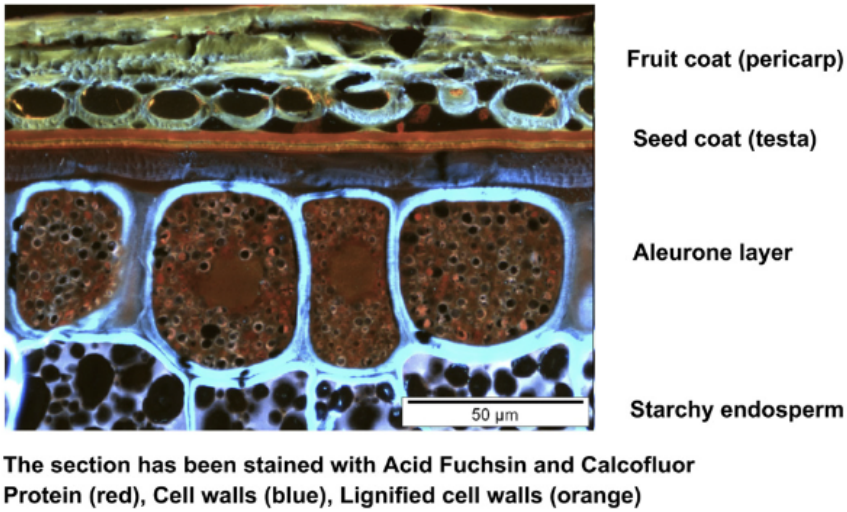

Figure 1.3. Light micrograph of the outer part of the grain of the wheat (bran and aleurone) From: Poutanen, (2012).

Endosperm is completed by starchy endosperm that represents the largest portion of the kernel and it is formed by cells that are packed with starch grains that are embedded in a protein matrix.

The cell walls of wheat starchy endosperm contain about 75% polysaccharides (comprising about 70% arabinoxylans, 20% beta-glucan, 7% beta-glucomannan and 3% cellulose). The principal features of endosperm cells are two main storage reserves: starch and protein. The proportional contributions of starch and matrix protein also vary according to cell position. All cells contain approximately the same mass protein, but the increasing starch content found toward the center of the caryopsis causes progressive dilution of other components as well as protein.

The last component of the kernel is the germ (or embryo). It is located on the dorsal side of the caryopsis and it is a very important structure because it contains the material necessary for the initialization of growth of a new plant. It comprises two major components, the embryonic axis and the scutellum. The latter is a protective layer

positioned between the endosperm and the embryo, in the form of a concave shield towards the endosperm (Swift & O'Brien, 1972).

1.3 Wheat composition

Carbohydrates account for about 80-85 % of the mature wheat grain (Shewry & Hey, 2015) as well as for other major cereals. Values reported for the amounts of individual groups in the wheat grain are in the order of about 1% or less monosaccharides (glucose, fructose) and disaccharides (sucrose and maltose), about 1% oligosaccharides (raffinose and fructo-oligosaccharides), 1-2% fructans, 65-75% starch and about 10% cell wall polysaccharides (mainly cellulose, arabinoxylan and β-glucan), the latter forming the major dietary fiber components (Lafiandra et al., 2014). However, there are large differences between the composition of the different grain tissues. In particular, the aleurone and outer layer (pericarp and testa), which form the bran fraction on milling of wheat, contain little starch and up to half of the dry weight is cell wall polysaccharides, while the starchy endosperm (the major storage tissue of the grain) comprises about 85% starch and only 2-3% cell wall polysaccharides.

Starch is present in the form of a mixture of large and small granules and it is comprised of two classes of glucose polymers, amylose and amylopectin, which vary in their proportions of α-1,4 and α-1,6 linkages and molecular weight.

Arabinoxylans (AX) comprises a backbone of β-D-xylopyranosyl (xylose) residues linked through (1→4) glycosidic linkages with some residues being substituted with α-L-arabinofuranosyl (arabinose) residues at either one or two positions. Some arabinose residues present as single substitutions on xylose may also be substituted with ferulic acid at the position five.

β-Glucan comprises glucose residues joined by β -(1→3) and (1→4) linkages. Single (1→3) linkages are usually separated by two or three (1→4) linkages.

Cellulose is a polymer formed by glucose molecule linked by β -(1,4) bonds that form long linear chains

Protein content can vary greatly in wheat, from as low as 6% to up nearly 20%. The level depends on wheat class, soil fertility of the growing region, and environmental conditions encountered during the growing season.

Osborn (1924) classified cereal protein into four classes depending on the solubility in different solvents: albumins soluble in water, globulins soluble in dilute salt solutions, gliadins soluble in aqueous alcohol solutions and the glutelins which are extracted using dilute acid or alkali. A further classification distinguishes proteins in two groups based on biological functions: storage and non-storage proteins. The starchy endosperm contains the major gluten storage proteins (gliadins and glutenins) and smaller amount of globulins proteins. Albumin and globulin, on the other hand, constitute the non-reserve proteins that are located in the germ and in the aleurone layer and include proteins that have important biological functions such as enzymes, nucleoproteins, membrane proteins and glycoproteins.

The nutritional quality of wheat grain is determined by the content of essential amino acids. These amino acids cannot be synthesized by human metabolism and hence must be provided with the diet. Comparison of the amino acid composition of wheat flour with the levels of essential amino acids recommended by the Food and Agriculture Organization of the United Nations (FAO) for infants and adults (FAO, 1970) shows that wheat is severely deficient in lysine, thus it is necessary to combine wheat with other sources of protein (such as animal protein or legume seeds) to provide a balance of essential amino acids (Shewry et al., 2009).

Lipids in wheat or wheat flour are minor constituent (1.5-2% of the caryopsis), they are distributed throughout the wheat kernel and represent components of membranes, organelles and spherosomes (membrane-bound droplets). Although the lipids are widely distributed in the kernel, they are minor constituents in all but one part – the germ.

The endosperm fraction contains a relatively low concentration of lipids (0.8-2.2%, d.w.), while the germ, which is 2-4% of kernel weight, contains a high concentration of lipids (28.5%, d.w.).

Next to the non-polar lipids (essentially triglycerides), the caryopsis contains polar lipids (glycolipids and phospholipids), whose functional properties (emulsifying, stabilizing) allow interactions with protein and starch molecules and are strategic for technological transformations. A part of the lipids is bound to starch to form starch-lipid complexes that influence the gelatinization and swelling behavior of the starch granules during cooking. The role played by polar lipids in determining the final volume of leavened baked products is important. It is believed that lipids are able to stabilize the gas microbubbles developed in the dough (Lafiandra & D'Egidio, 2014). The significant presence of unsaturated and poly-unsaturated fatty acids in wheat lipids could cause deterioration phenomena, such as rancidity. This explain why during milling of wheat, the germ is separated to ensure preservation of the final products, despite lowering nutritional values.

Vitamins are organic compounds required in small amounts for the maintenance of normal biochemical and physiological functions of a human system and they are considered essential nutrients because we are not able to synthesize them in sufficient amounts to meet our needs and requirements. Based on their solubility, vitamins are

divided in lipid-soluble vitamins A, D, E and K, and water-soluble vitamins such as B group vitamins, vitamin C, choline and myoinositol.

Wheat is generally acknowledged to be a good source of several B vitamins, especially thiamin, niacin, vitamin B6, and folate. B vitamins are known to be unevenly distributed in wheat kernel. The aleurone layer provides about 80% of the niacin, 60% of the vitamin B6 and 32% of the thiamin, as well as a significant proportion of each of the other vitamins. Thiamin is virtually confined to the scutellum and aleurone layer. An important group of vitamins associated with lipids is represented by tocopherols and tocotrienols which are collectively called tocols or vitamin E. Most of tocopherols are concentrated in the wheat germ, while tocotrienols are concentrated in the pericarp, testa and aleurone (Lampi et al., 2008).

Minerals represent the fraction of a food that is referred to as "inorganic" nutrients. Wheat is a rich source of many minerals and trace elements. The ash content of wheat can vary from 1.17 to 2.96%. This variation is caused by genotype, wheat class, cultivar as well as the growing location and year. Minerals and trace elements of wheat are mostly situated in the outer part of the grain. However, the bioavailability of minerals and trace elements from wheat and wheat products is associated with dietary fiber, which has potent cation-exchanging capacity and may therefore have a negative effect on the bioavailability of minerals. In particular, poor availability is associated with the presence of phytic acid that is a potent mineral chelator (Piironen et al., 2009).

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Chapter 2: STARCH

2.1 Introduction

Starch is the main storage carbohydrate in wheat and constitutes about 60–75% of grain and 70–80% of flour. It plays an important role in human nutrition as it contributes to >50% of caloric intake in Western world and up to 90% in developing countries (Wang et al., 2015). Native starch is highly variable in structure and functionality between and within plant species, and even from the same plant cultivar grown under different conditions. This variability is evident in granule morphology (size, shape), amylose content, amylopectin architecture (chain length distribution and placement of branches), and the way these two macromolecular constituents are arranged into crystalline and amorphous regions within granules (Wang & Copeland, 2013).

2.2 Composition

Starch consists of two glucose polymers, namely amylose and amylopectin. Amylose is essentially a linear polymer in which glucose residues are α-D-(1-4) linked, amylopectin is a larger branched molecule with α-D-(1-4) and α-D-(1-6) linkages (Sajilata et al., 2006). Starches are classified according to the amylose/amylopectin ratio. Those starches with 25-30% amylose and 70-75% amylopectin are usually named as “normal” starches; some starches do not contain or contain negligible amount of amylose are called “waxy” starches; a third group includes starches with contents of amylose significantly higher (50-70 %) known as high amylose starches.

Amylose has a polymerization degree (DP) up to 5000, the chains can easily form single or double helices. Amylopectin is highly branched with ~6% of α-1,6 linkages, compared to amylose which has few branches (< 1%), and it has a polymerization degree (DP) from 5000 to 100,000. Individual branches of amylopectin are shorter

with an average DP of 17–25 compared to amylose in which the branches are significantly longer with an average DP of 10^3-10^4 (Bird & Regina, 2018).

The molecular structure of amylopectin is considerably more complex than amylose. The organization of the unit chains in amylopectin is critical for the understanding of the structure and architecture of the entire macromolecule. At present, the actual organization is not clarified. A hypothesis is the cluster model (Figure 2.1) which suggests that the short unit chains are organized into clusters and the long chains interconnect them (Vamadevan & Bertoft, 2014). Chains that do not carry other chains through (1,6)-linkages are known as A-chains, whereas chains that carry other chains are B-chains. B1 chains are present within single clusters, whilst B2 and B3 chains are long chains interconnecting a number of clusters.

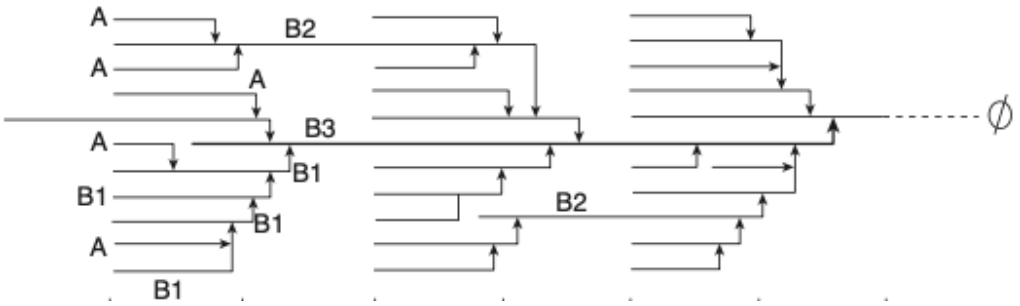

Figure 2.1. A cluster model of amylopectin with A, and B1–B3 chains. From: Jane, (2005)

Wheat starch occurs in the form of semi-crystalline granules, which have a very complex hierarchical structure. The granules possess generally rings, or shells, commonly known as “growth rings”, with alternating amorphous and semi-crystalline structures and typical thickness of 100–400 nm (Figure 2.2). Amylose is found in the amorphous lamella of the starch granule, while amylopectin is responsible for the crystalline lamella of the granule, and its branching points are part of the amorphous

lamella. The presence of amorphous and crystalline lamellae in the starch granule confers this biopolymer a semi-crystalline entity (Bello-perez et al., 2018).

Figure 2.2. Stacked amorphous (a) and crystalline (c) growth rings.

Different size and shapes of starch granules build up during the development of grain. The larger "A" granules have a lenticular shape and a diameter from 15 to 30 μm, whereas "B" granules have a diameter that is typically below 10 μm and blocklike structures that range from polyhedral to near-spherical. A third class (C) exists and it is characterized from very small granules (2-3 μm) (Bechtel et al., 1990).

The A-, B- and C-granules differed according to the time of biosynthesis during grain filling. The biosynthesis of A-granules starts 4 days after anthesis (DAA) with granule growth and development continuing over the next 20 days, while that of B-granules initiated 10–12 DAA with granule growth beginning 18–20 DAA (Wei et al., 2010).

Wide variation in granule size distribution exists among starches from different wheat type and cultivars/varieties (Figure 2.3).

The starches from hard wheat varieties contain higher proportion of B-granules and lower of A-granules than soft wheat. Normal and waxy starches contain both A- and B-granules, however, these differ in relative granule morphology, the starch from waxy cultivars/varieties showed more spherical disc-like granule morphology (Shevkani et al., 2016).

Environmental conditions have significant effects on granule size distribution. Low temperatures during grain development extend grain-filling period, providing more time for the synthesis and development of B- and C-granules, leading to higher proportion of small granules. In contrast, high temperatures accelerate grain development and cause premature maturation, limiting the opportunity for B- granule initiation and growth and thus suppressing B- granule numbers (Stone & Morell, 2009).

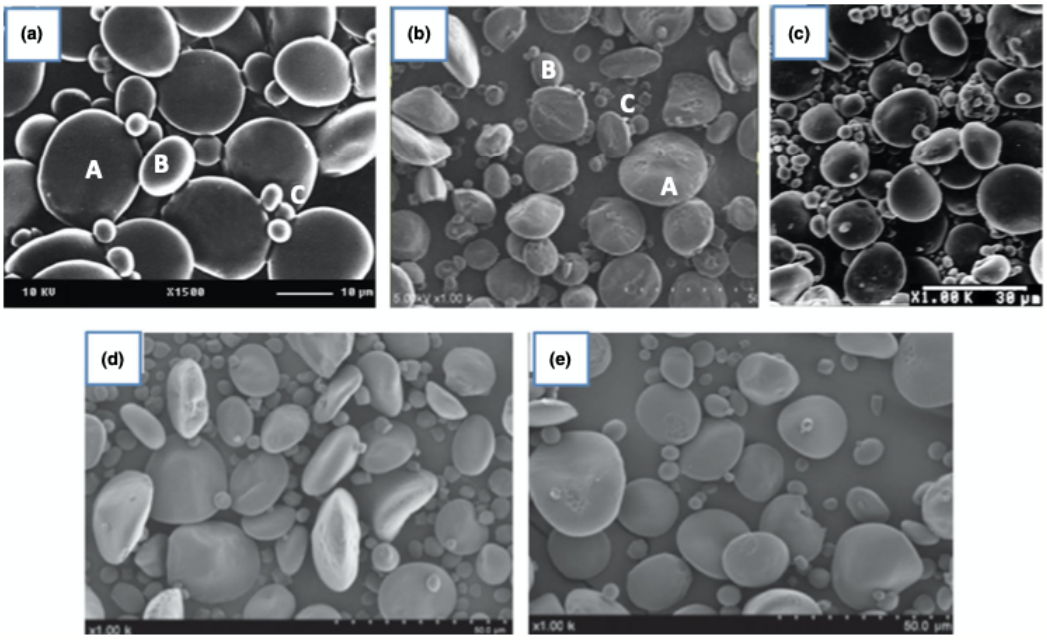

Figure 2.3. Scanning electron micrographs of starches from different wheat types: (a) normal, (b) waxy, (c) high-amylose, (d) hard and (e) soft wheat. From: Shevkani et al. (2016).

2.3 Starch biosynthesis

Starch biosynthesis in the cereal endosperm requires the coordinated activities of several enzymes, including adenosine 5'diphosphate-glucose (ADP-Glc) pyrophosphorylase (AGPase), granule-bound starch synthase (GBSS), soluble starch

synthase (SS), starch branching enzyme (BE) and starch debranching enzyme (DBE) (Figure 2.4). AGPase catalyzes the first key regulatory step in the starch biosynthetic pathways present in all higher plants that produce ADP-Glc and pyrophosphate (PPi) from Glc-1-P and ATP. AGPase activity is localized at both the plastids and cytosol of cereal endosperm (Jeon et al., 2010). Starch synthase operates in the elongation of linear glucan chains by catalyzing the transfer of the glucosyl unit of ADP-Glc to the non- reducing end of a glucan chain.

A single starch synthase, the granule bound starch synthase (GBSS), is involved in the synthesis of amylose. This enzyme is also known as waxy protein reflecting the typical phenotypes of seeds lacking amylose. The inactivation of genes encoding GBSS results in starch containing only amylopectin (Lafiandra et al., 2014).

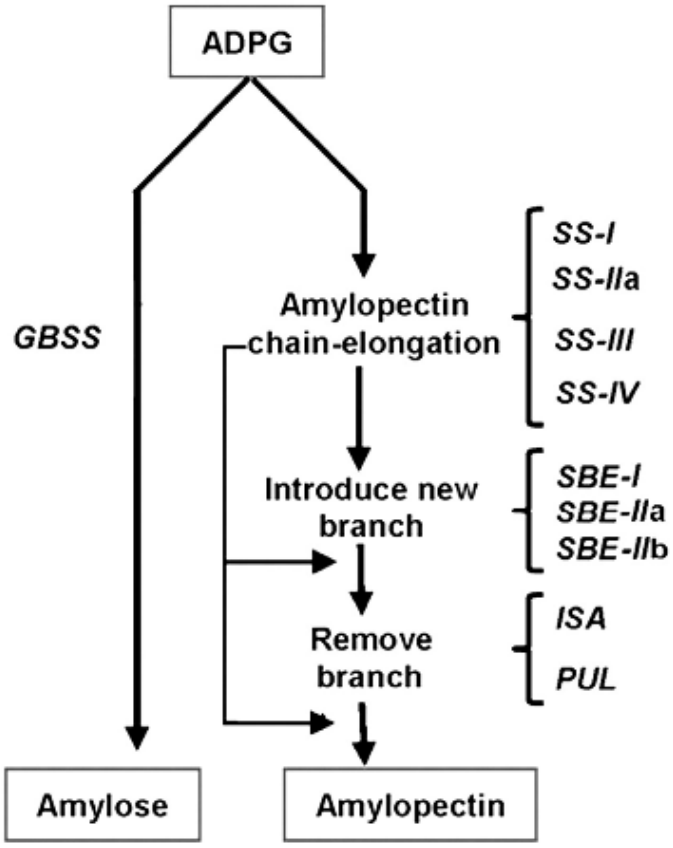

Figure 2.4. Pathway of starch biosynthesis in wheat. From: Lafiandra et al. (2014).

Amylopectin synthesis is more complex than the amylose synthesis and involves several different enzymes. Four classes of starch synthase (SSI, SSII, SSIII and SSIV) have been shown to contribute to amylopectin synthesis. SSI is primarily responsible for the synthesis of the shortest chains that are those with a degree of polymerization (DP) of 10 glucosyl units or less. Further extension to produce longer chains that extend between clusters is catalyzed by SSII and/or SSIII (James et al., 2003). Two different types of SSII genes are present in cereals, with one being expressed in the endosperm (SSIIa) and the other in the leaf (SSIIb). Little is known about the contributions of SSIV isoforms to glucan chain lengths in cereals (Jeon, 2010).

Branching of the glucan chains is catalysed by starch branching enzymes, through cleavage of the α-1,4 glucosidic linkage of a glucan chain and subsequent bonding of the cleaved portion of this glucan chain to an adjoining chain through an α-1,6 linkage (Keeling & Myers, 2010). There are two classes of BE (BEI and BEII) that differ in terms of the lengths of chains, with BEII transferring shorter chains than BEI. In cereals, there are two closely related forms of BEII (BEIIa and BEIIb). These also differ in chain-length specificity, with BEIIb transferring shorter chains than BEIIa (James et al., 2003).

Starch synthesis involves also debranching enzymes (DBEs) in addition to SSs and BEs. Two DBE families exist in plants, isoamylase-type and pullulanase-type. Both types hydrolyze α-1,6 linkages, but they differ in substrate specificity. Several models could explain the function of DBE in starch synthesis. The DBEs directly participate in amylopectin synthesis, selectively removing branches that are inappropriately positioned (Nakamura, 2002). Accordingly, DBE activity would be required for maintenance of the cluster structure of amylopectin, for the dense packing of linear chains and for growing chains to crystallize onto the granule surface.

2.4 Starch functionality

Starch influences the characteristics of many foods, contributing to control moisture, viscosity, texture, consistency, mouth-feel and shelf-life during processing and in finished products (Wang & Copeland, 2013). The changes of functional properties of starch involve water uptake, granule swelling, formation of a viscoelastic paste during heating, followed by reassociation of dispersed starch chains on cooling and formation of a gel.

When heated in excess water, starch granules become hydrated, swell and undergo an irreversible phase transition, referred to as gelatinization. Starch gelatinization has been broadly defined as the "collapse (disruption) of molecular orders (breaking of hydrogen bonds) within the starch granule manifested in irreversible changes in properties such as water uptake, granular swelling, crystallite melting, unwinding of double helices, loss of birefringence, starch solubilization and viscosity development" (Wang & Copeland, 2013) (Figure 2.5).

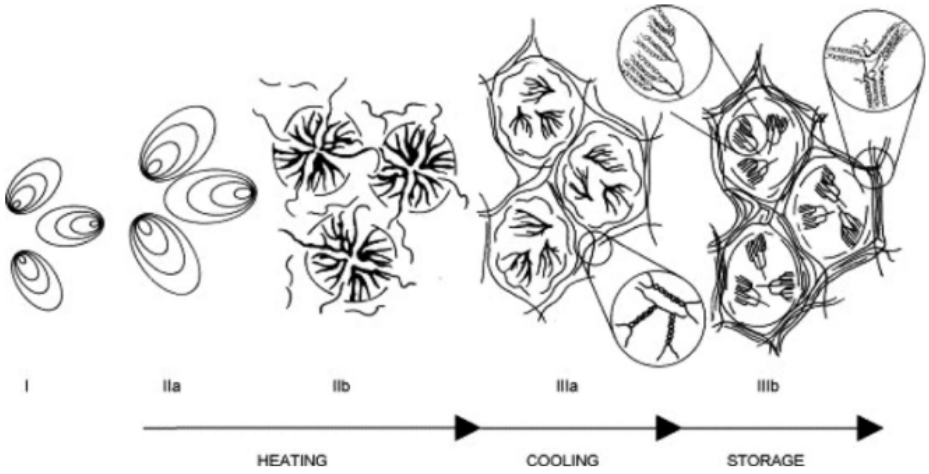

Figure 2.5. Schematic representation of changes that occur in a starch–water mixture during heating, cooling, and storage. From: Wang et al. (2015).

On heating, water firstly enters the amorphous regions, which expand and transmit disruptive forces into the crystalline regions. These changes are accompanied by swelling of the granules, which under mixing conditions results in an increase in viscosity before the eventual collapse of the granules to form a paste, if the water content of the system is high enough (Wang et al., 2020).

An increase in gelatinization temperature of starch is attributed to the preferential melting of less stable crystallites during the thermal processing, leaving more stable residual crystallites with a higher melting temperature. The disruption of starch crystallites increases the proportion of amorphous regions in starch, which may also reduce access of water to the residual crystallites, thus leading to increased gelatinization temperatures (Liu et al., 2022).

The dissociated amylose and amylopectin chains in the gelatinized starch paste can re-crystallize gradually on storage into a different ordered structure in a process referred to as retrogradation, in particular amylose molecules reassociate to form double helices, while the outer branches of amylopectin align themselves to form crystallites. Starch retrogradation is usually accompanied by a series of physical changes such as increased viscosity and turbidity of pastes, gel formation, exudation of water and increased degree of crystallinity with the appearance of B-type crystalline polymorphs (Hoover et al., 2010). Retrogradation is an ongoing process, which initially involves rapid recrystallization of amylose molecules, amylopectin, on the other hand, reassociated slowly during storage. Therefore, waxy and normal starches showed slower and lesser retrogradation than high-amylose starches. Amylopectin recrystallization related positively to amylopectin chain length as the starches with higher proportion of short chains (DP 6-9) were less susceptible to retrogradation than that with higher proportion of chains with DP 14-24. The starches with long

amylopectin chains had sufficient glucose units to form double helices during storage of gelatinized starches, hence showed higher rate of retrogradation. Amylose-based networks are considered to provide starch gels with elasticity and strength against deformation, whereas soft gels containing aggregates in the absence of networks (waxy starch pastes) display easier penetrability and greater stickiness and adhesiveness. Retrogradation is also influenced by the presence of other minor constituents of starch, like lipids and proteins. These play important roles in slowing down and retarding retrogradation, because the association of starch lipids and proteins with amylose and outer chains of amylopectin during gelatinization inhibits amylose aggregation and formation of double helices between amylose molecules and amylopectin recrystallization (Shevkani et al., 2016). Starch retrogradation is a subject of great interest to food scientists and technologists because it has a profound effect on starchy foods, in particular it is considered to be an undesirable process that occurs during the storage of baked products (Liu et al., 2022).

The changes that occur in a starch suspension are widely quantified using a Rapid Visco Analyser (RVA) under controlled heating, cooling and stirring conditions. During a cycle of heating and cooling, the RVA trace provides information on pasting temperature, the peak viscosity, breakdown (stability of hot paste to shear forces), setback (initial retrogradation of the starch paste on cooling) and the final viscosity.

A typical pasting profile of wheat starch is shown in Figure 2.6.

During heating of starch:water mixture, the viscosity increases due to the swelling of granules at a specific temperature value referred to as pasting temperature (PT). After reaching the maximum value, the viscosity starts decreasing as the granules rupture and starch components leach and disperse in the aqueous phase. The decrease in viscosity (breakdown viscosity; BV) is a measure of the resistance of swollen granules

towards disintegration at high temperature. With cooling, the retrogradation occurs and the viscosity of starch pastes increases due to amylose and amylopectin reorganization.

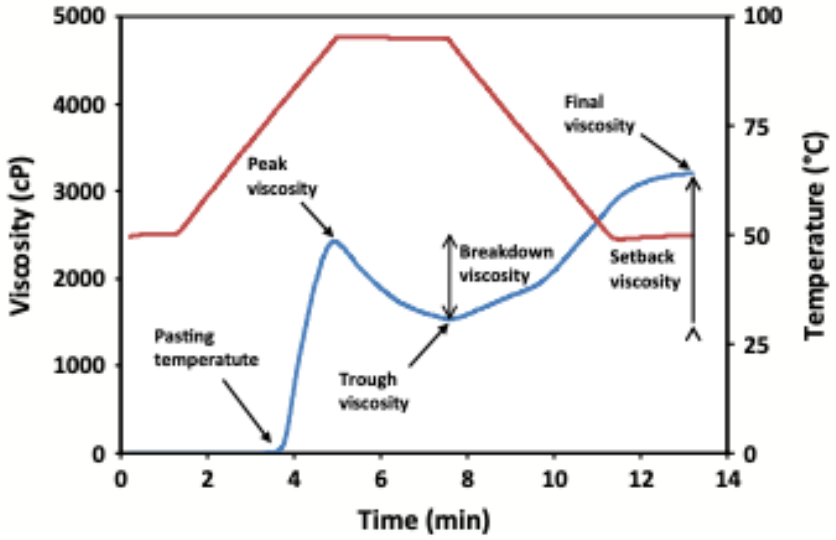

Figure 2.6. Typical pasting profile of wheat starch. From: Shevkani et al. (2016).

As opposed to low amylose starches whose curves run in a very low viscosity region and their viscous properties do not really change during the analysis time (Jaksics et al., 2020), waxy starches are usually known to have greater peak viscosities followed by a more noticeable breakdown and a lesser degree of setback during pasting (Hsieh et al., 2019).

2.5 Starch digestibility and resistant starch

Starch digestion in humans occurs in the gastrointestinal tract, where salivary and pancreatic α-amylases break the starch molecules into smaller oligosaccharides, maltose, maltotriose and other branched oligosaccharides, which are further converted into glucose in the small intestine (Magallanes-cruz et al., 2017).

The morphological characteristics of starches influence digestibility. Concerning granules size, there is a direct negative relationship between large size granules and starch digestibility. The lower susceptibility of the large granule starches to enzymatic hydrolysis has been suggested to be due to their smaller granule specific surface area, which may decrease the binding capacity of enzymes resulting in a lesser hydrolysis than small granules (Singh et al., 2010).

Another morphological characteristic that might impact enzymatic digestion is the surface of starch granules. A higher digestibility was encountered for cereal starches compared to tuber and legume starches that may be attributed to the presence of numerous pinholes on the surface layer and pores that penetrates towards the interior of the cereal granules and facilitate the entry of the amylases for digestion (Dreher et al., 1984).

Amylose/amylopectin ratio also influence digestibility: raw starches high in amylopectin have been shown to be digested more quickly than those high in amylose. Amylopectin has a much larger surface area per molecule than amylose, which makes it a preferable substrate for amylases attack, in addition amylose chains are more bound to each other by hydrogen bonds making them less available for amylolytic attack than amylopectin which is highly branched (Singh et al., 2010).

Predicting and controlling the glucose absorption and thus the glycemic index due to ingestion of starchy food is of great interest in the context of worldwide health concerns (Singh et al., 2010).

Starch digestibility is assessed by *in vitro* or *in vivo* methods. *In vivo* tests have proved to be difficult and expensive because they demand several subjects for a long period of time and the facilities necessary for this kind of study are not always present in food research laboratories. Several *in vitro* assays have been proposed to evaluate the rate

of starch hydrolysis, which is considered to be a predictor of the physiological effects of foods. An example is an *in vitro* assay proposed by Goñi et al. (1997) that uses proteolytic enzymes in combination with amylases, in contrast with other assays that only use amylases. Englyst et al. (1992) developed an *in vitro* method to classify starch into three types based on its digestion rate in the small intestine: **rapidly digestible starch (RDS), slowly digestible starch (SDS)** and **resistant starch (RS)**.

RDS consists mainly of amorphous and dispersed starch and is found in high amounts in starchy foods cooked, such as bread and potatoes. It is measured chemically as the starch, which is converted into glucose molecules in 20 min of enzyme digestion. **SDS** is completely digested in the small intestine, but it is digested more slowly. This category consists of physically inaccessible amorphous starch and raw starch. It is measured chemically as starch converted to glucose after a further 100 min of enzyme digestion. **RS** is the term used to describe that portion of starch that escape digestion in the small intestine in healthy individuals (Sajilata et al., 2006).

Resistant starch is subdivided into five categories RS1, RS2, RS3, RS4, RS5 (Birt et al., 2013).

RS1 is the physically inaccessible starch, which is entrapped within whole or partly milled grains or seeds. Starch granules are surrounded by protein matrix and cell wall material that provide a physical barrier, preventing enzymes from reaching and hydrolyzing the starch. In addition, during cooking the thick cell wall of legume seeds and the protein matrix in cereal grains prevent water penetration into the starch and thus the swelling of the granules. Therefore, the starch is not readily susceptible to enzymatic hydrolysis

RS2 represents starch that is in a certain granular form and resistant to enzyme digestion. Examples of RS II are uncooked potato starch, green banana starch and

high-amylose maize starch, which display the B- or C-type polymorph. With cooking, most of the starch, such as that in baked potato, becomes highly digestible as a result of starch gelatinization. An exception is high-amylose starch which has substantially longer branch chains and a larger proportion of amylose that increase gelatinization temperature and thus after boiling or cooking at a temperature below its gelatinization temperature, this type of starch retains its crystalline structure and remains resistant to enzymatic hydrolysis.

RS3 is retrograded starch, which may be formed in cooked foods that are kept at low or room temperature. After starchy foods are stored, particularly in a refrigerator, amylose molecules and long branch chains of amylopectin form double helices that do not fit into the enzymatic binding site of amylase, thus they cannot be hydrolyzed by this enzyme.

RS4 describes a group of starches that have been chemically modified and include starches which have been etherised, esterified or cross-bonded with chemicals in such a manner as to decrease their digestibility, because they lose the ability to swell during cooking. Consequently, the highly cross-linked starch remains in a granular form after cooking, with little enzymatic susceptibility, and cannot be hydrolyzed by amylases.

RS5 is referred to starch that interacts with lipids forming single-helical complexes difficult for enzymes to access.

2.5.1 Beneficial effects of resistant starch on health

RS has received much attention for its potential health benefits. Resistant starch is one of the most abundant dietary sources of non-digestible carbohydrates and could promote large bowel health and preventing bowel inflammatory diseases (IBD) and

colorectal cancer (CRC) as well as positively impact on glucose metabolism (Fuentes-Zaragoza et al., 2010).

The most important beneficial effects of RS are attributed to its prebiotic potential, since it escapes digestion in small intestine and reaches the large bowel, where it can serve as a substrate for the selective growth of probiotic bacteria such as lactobacilli, bifidobacteria and streptococci, which are known to be beneficial and might enhance human health (Magallanes-cruz et al., 2017).

From their fermentation, they are able to produce microbial metabolites such as short chain fatty acids SCFA (acetate, propionate and butyrate) that have several physiological and metabolic impacts on human health (Ríos-Covián et al., 2016). Among the beneficial effects of SCFA there is the reduction of the luminal pH, which inhibits pathogenic microorganisms and increases the absorption of nutrients (Macfarlane & Macfarlane, 2012). In particular, butyrate is one of the main energy substrates for large intestinal epithelial cells (colonocytes) and in cell culture, butyrate has antitumorigenic properties, including reducing cell proliferation and inducing apoptosis of colorectal tumor cell lines; this makes fermentable RS fractions interesting in preventing colon cancer (Fung et al., 2012).

Foods containing RS moderate the rate of digestion. The slow digestion of RS has implications for its use in controlled glucose release applications. Consequently, replacing ordinary dietary starch with resistant starch contributes to diabetes management. It has been reported in human studies that consuming high amylose foods significantly reduced blood glucose concentrations (Ang et al., 2020; Belobrajdic et al., 2019).

Resistant starch has also been shown to promote satiety increasing secretion of the putative satiety hormones GLP-1 and PYY (Birt et al., 2013) and therefore reduce food intake aiding control of weight.

It is worth noting that although RS provides health benefits, most RS shows low thermal stability during food processing. For instance, cooking native cereal starches diminishes RS content and increases RDS content due to the disorganization of the starch structure, which also increases enzymatic susceptibility. For that reason, starch modification has been extensively studied as an alternative approach to improve the slow and resistant digestion properties of starch.

2.6 Strategies to modify wheat starch characteristics

Different approaches have been used to modify structural/compositional characteristics of starch.

-Classical plant breeding is based on exploiting natural variation, which is generated by recombination during crossing of genotypes with different genetic backgrounds, for example between a crop and a wild relative. However, an extensive program of back crossing is also required to eliminate deleterious genes from the wild relative which are not related to the trait of interest (Lafiandra et al., 2014).

-Transgenesis is increasingly being applied to improve grain composition and quality in this issue.

Regina et al. (2006) used RNA interference (RNAi) gene silencing to suppress simultaneously the expression of a target gene. In particular, RNAi technology was used to silence the expression of SBEIIa and SBEIIb, resulting in a high-amylose (>70%) phenotype. However, the main barrier to the deployment of transgenesis to

improve the health benefits of cereal grain is low acceptability by consumers and regulatory authorities (Botticella et al., 2011).

-Mutation breeding, which is based on the use of radiation or chemical agents to induce mutations, has proved to be very effective in generating novel genetic diversity for important traits that has been exploited in breeding programs.

Recent developments in sequence-level detection coupled with the availability of large genomic resources and bioinformatic tools have led to the development of a technology termed TILLING (Targeting Induced Local Lesions in Genomes).

This approach is based on identification, by polymerase chain reaction (PCR), of single base nucleotide polymorphisms (SNPs) in specific genes within a population of plants, produced by treating seeds or pollen with a chemical mutagen (Sestili et al., 2010). A major advantage of TILLING is that chemical mutagens, such as EMS (ethylmethane sulphonate), sodium azide and MNU (N-methyl-N- nitrosourea), are able to alkylate nucleotides to induce point mutations which are randomly distributed over the entire genome. Treatment of seeds with mutagens is very effective in producing nonsense, missense and silent mutations. Moreover, this approach is particularly suitable for polyploid species with complex genomes such as bread wheat that has three different genomes.

Slade et al. (2005) identified a total of 246 novel waxy (GBSSI) alleles in durum and bread wheat and crossed null mutants in different homoeologues to produce a waxy phenotype. Similarly, Sestili, et al. (2010) identified increased allelic variation present in the three homoeoloci of the SSIIa gene by analyzing a mutagenised population of the bread wheat cultivar Cadenza, using a combination of forward genetics and TILLING.

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Chapter 3: OUTLINE OF THESIS

Wheat is one of the most cultivated, consumed and traded grains with a worldwide diversity of thousands of cultivars/varieties with high diffusion in the cuisines, cultures and culinary histories of many countries.

Wheat kernel is a concentrated source of dietary fibre as well as various minerals and vitamins, and numerous phytochemicals. However, modern milling and food processing practices have had an adverse effect on the fibre profile and content, and potential health benefits, of contemporary wheat- based foods. Thus, the main component in extensively processed wheat foods is starch and it is mainly composed of digestible carbohydrates.

Novel wheat varieties that have a markedly elevated amylose content are characterized by a higher resistant starch fraction which is included in the definition of dietary fiber, and can be conveyed to both wholegrain and refined flours providing optimal raw materials to produce low-glycemic index foods. However, altered starch properties reflect changes in grain quality traits and chemical composition, thus the assessment of the technological quality of high amylose flours is important to obtain a final product with structural and sensorial properties comparable to conventional products.

The research project aims to 1) evaluate chemical, physical and technological differences between two grain samples (*Triticum aestivum* L.): a high amylose wheat genotype obtained, using TILLing procedure, from the normal starch wheat genotype that was used as reference control; 2) determine milling performance with the application of conventional soft-wheat milling and a hard-wheat mill coupled with debranning; 3) define chemical properties and technological/rheological aptitudes of the obtained flours; 4) produce end products replacing conventional wheat flours with the obtained high amylose flours and finally, 5) evaluate nutritional, health and sensorial properties of the innovative products.

Chapter 4: MILLING AND RHEOLOGICAL PROPERTIES OF HIGH AMYLOSE WHEAT

De Arcangelis, E., Trivisonno, M.C., Angelicola, M., Quiquero, M., Di Nardo, V., Falasca, L., Sestili, F., Messia, M.C., Marconi, E. 2021. Milling and rheological properties of high amylose wheat. *Journal of Cereal Science*, 102, Article number. 103335, ISSN: 0733-5210.

Abstract

The technological quality of a high amylose bread wheat variety (amylose/amylopectin 1:0.7) was explored performing different milling protocols, with subsequent evaluation of the chemical composition and rheological properties of the milling fractions and recombined flours. High amylose wheat yielded a lower amount of flour (<50%) and less refined products compared to control line. Grain debranning improved flour yields, releasing more refined reduction products. The milling of high amylose wheat with a hard-wheat diagram enhanced flour yield and refining rate of products, also giving a semolina type flour that can be employed e.g. for the production of pasta and couscous. The high amylose flours are characterized by relevant levels of resistant starch (27% of total starch on average) that make them suitable for the production of functional foods in agreement with the EU Regulation 432/2012. In addition, from the experimental results, it was put in evidence that official protocols for rheological analysis of flours (gluten index, alveograph and falling number) should be adapted for high amylose wheat samples, due to the impact that the different starch composition has on dough properties, specifically on water absorption and viscosity. Overall, rheological features of high amylose wheat flours exhibited higher water absorption, stability, and altered starch pasting properties.

Key words*: milling performance, dough rheology, high amylose flours, resistant starch*

4.1 Introduction

Starch is the main component of endosperm in wheat kernel and it is composed of two polymers: amylose and amylopectin. Amylopectin is a large highly branched molecule of α-D-glucopyranosyl unit (1–4) linkages with 5% (1 → 6)-α linkages. Amylose is mainly a linear chain of (1 → 4)-linked α-D-glucopyranosyl units.

Usual starch composition in wheat consists of 25% amylose and 75% amylopectin, however genetic approaches targeting starch-synthetizing enzymes produced waxy wheats, containing up to 100% amylopectin, and high amylose wheats that instead exhibit a higher proportion of amylose (Hung et al., 2006). Amylose-enriched starches enhance the nutritional profile of wheats, generating a higher fraction of resistant starch (RS), which is defined as the proportion of starch that cannot be digested by amylases in the small intestine, but only fermented by gut microbiota, promoting benefits to gut health and improving glucose metabolism after a meal (Birt et al., 2013). High amylose starches (amylose up to >70% of total starch content) were obtained in wheats (Botticella et al., 2018; Regina et al., 2015; Schönhofen et al., 2017; Slade et al., 2012), leading to a proportional increase of RS in flours up to > 10% (Regina et al., 2015); these products were successful employed by different authors to obtain high resistant starch foods (Ang et al., 2020; Newberry et al., 2018). Furthermore, within the European legislative framework, a specific health *claim* regarding RS is enlisted in EU Regulation 432/2012, prescribing a content of resistant starch of at least 14% of total starch in a product to *claim* the physiological effect of improved glucose metabolism after a meal. Together with the nutritional enhancement of high amylose grain, researches highlighted profound effects of genetic manipulation on the technological quality of these genotypes. High amylose wheat kernels are characterized by an increase in grain hardness (Botticella et al., 2018; Hogg et al.,

2017), decreased kernel weight and test weight (Botticella et al., 2016; Hogg et al., 2017; Slade et al., 2012), reduced starch content (Hogg et al., 2017; Slade et al., 2012) and modifications of size and morphology of A- and B- type starch granules (Hogg et al., 2017; Slade et al., 2012). Few researches were conducted on the milling performance of high amylose soft wheats, which evaluated only conventional grinding (Hogg and Giroux (2019)), so to fill these gaps, this study was developed performing different milling trials calibrating tempering in combination with debranning. On the selected flours, rheological properties were evaluated to define processing aptitude of high amylose flours for the production of foods with improved nutritional profile.

4.2 Materials and methods

4.2.1 Wheat samples

The bread wheat cultivar Cadenza (normal starch wheat, NSW) and a derived high amylose line (HAW) (Botticella et al., 2018) were grown in field during the season 2018–2019 at the Experimental Farm "Nello Lupori" (University of Tuscia) located in Viterbo, Italy (lat. 42°26' N, long. 12°04′ E, altitude 310 m a.s.l.). Crop management was performed using standard cultivation methods. Grain samples were milled with a laboratory mill (Laboratory Mill 3100, Perten Instruments) for analysis.

4.2.2 Grain characteristics

Test weight (kg/hL) was measured with a Shopper chondrometer equipped with a 250 mL cylinder. Kernel weight and hardness were determined using Perten SKCS 4100 (Perten Instruments, Sweden).

4.2.3 Grain debranning

Lab tests of debranning were performed with a laboratory debranner (Taka Yama mod. TM-05, Taiwan Province, China). Aliquots of high amylose and normal starch wheat kernels (100g) were debranned removing from 5 to 30% of outer layers in order to choose the most suitable percentage of debranning. Debranning trial with a pilot equipment was performed with NAMAD debranner (NAMAD Impianti, Rome, Italy).

4.2.4 Grain milling with soft-wheat mill

The same milling protocol was firstly applied to wholegrains (HAW and NSW) using a soft-wheat mill (NAMAD SG2000, Rome, Italy), equipped with three break rolls, three reduction rolls and six steel screens (Figure 4.1). Aliquots (5 kg) were tempered until reaching a final moisture of about 17%. Water was added and mixed with kernels to allow an adequate distribution on the surface, then wheat was stored for 12 h and then milled. Three breaking rolls flours (B1, B2, B3) and three sizing rolls fractions (C1, C2, C3), from break and reduction rolls respectively, and bran and shorts, which are by-products, were obtained from milling (Milling trial 1 – MT1) (Figure 4.3 a-b). To evaluate the effects of reduced hydration of the kernel, a portion of HAW was also tempered (13% of final moisture) and milled (Milling trial 2 – MT2) (Figure 4.3 c). HAW was also debranned at 6% (on the basis of debranning tests) to evaluate the reduction of ash content and milled without previous tempering (Milling trial 3 – MT3) (Figure 4.3 d). Flour yields were expressed as a percentage of initial product.

4.2.5 Grain milling with hard-wheat mill

HAW debranned at 6% was tempered (approximately 14% moisture) and then milled (Milling trial 4 – MT4) (Figure 4.3 e) in a MLU-202 mill (Bühler, Uzwill, Switzerland) equipped with three break rolls, three reduction rolls and six steel screens (Figure 4.2 a), obtaining three breaking rolls flours (B1, B2, B3) and three sizing rolls fractions (C1, C2, C3) from break and reduction rolls respectively, semolina, which was refined using a small scale purifier (Figure 4.2 b), and bran and shorts, which are by-products. Flours and semolina yields were expressed as a percentage of initial product.

4.2.6 Flours selection

To study chemical and rheological properties of high amylose flours, milling fractions were recombined taking into account high yield, thus including only high amylose flour obtained from application of debranning, and selecting fractions with ash content lower than 0.80% d.w.

The flours chosen were the following:

- reduction releases (C1, C2, C3) (high amylose flour, HAF) obtained from high amylose wheat milled with a soft-wheat mill (HAW – MT3) (Figure 4.3 d);
- high amylose semolina-type flour (high amylose semolina, HAS) obtained from high amylose wheat milled with a hard-wheat mill (HAW – MT4) (Figure 4.3 e);
- the mix of releases B2 and C1 (HAF(B2C1)) obtained from high amylose wheat milled with a hard-wheat mill (HAW – MT4) (Figure 4.3 e);
- the flour obtained by NSW including both breaking and reduction products (NSF) used as a control (NSW – MT1) (Fig. 4.3 a).

All the fractions were mixed with the same proportion of milling yields.

Figure 4.1. Soft-wheat mill (NAMAD SG2000)

a)

b)

Figure 4.2. (a) Hard wheat mill (Bühler MLU-202). (b) Small scale purifier

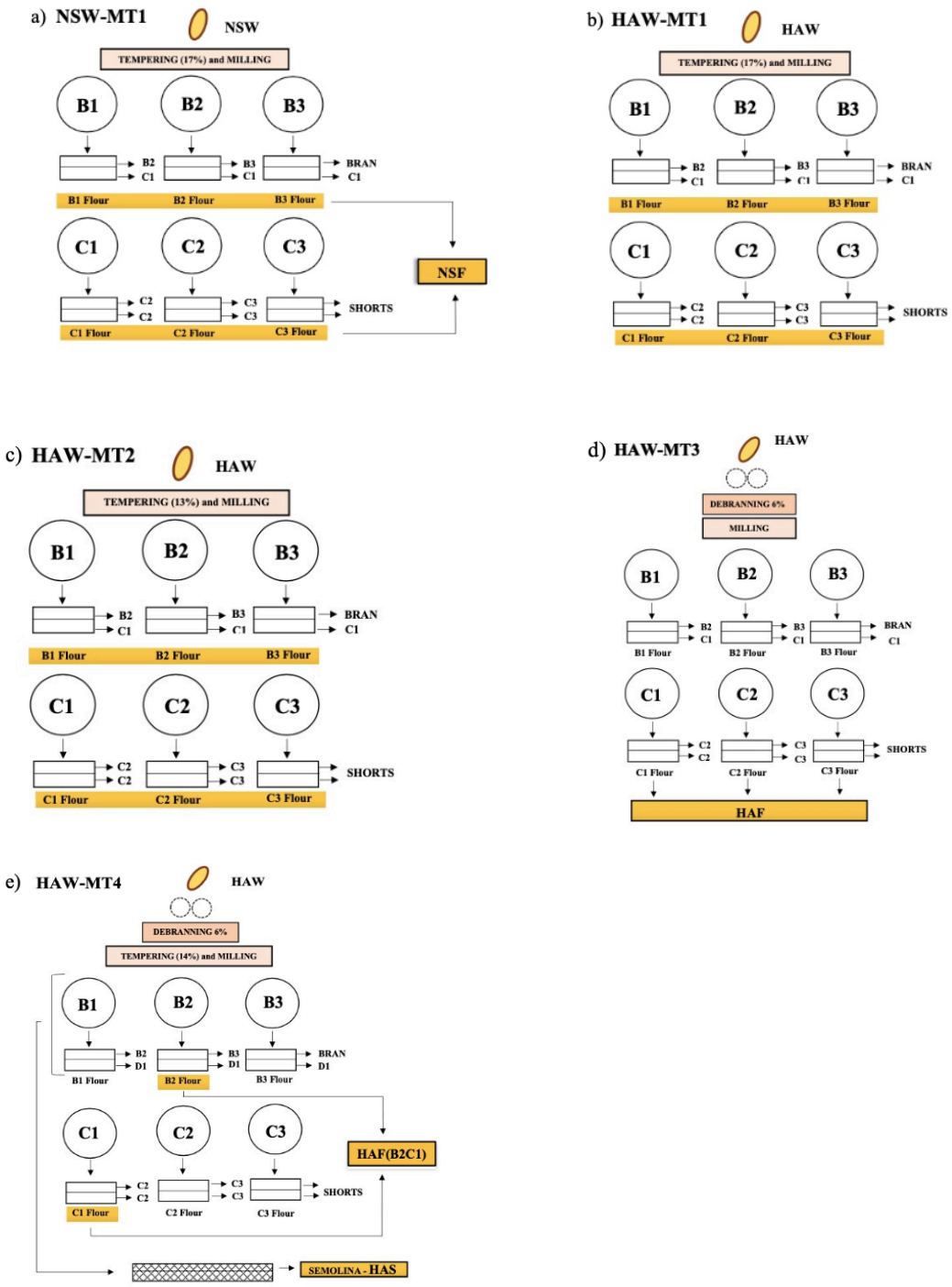

Figure 4.3. Schematic representation of milling diagrams and selected flours. B1, B2, B3 couples of breaking rolls. C1, C2, C3 couples of reduction rolls. HAW, high amylose wheat; NSW, normal starch wheat; MT, milling trial

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4.2.7 Chemical analysis

Moisture was determined according to ICC methods 110/1 (ICC, 1995). An aliquot of about 10 g of sample is accurately weighted in a moisture glass bowl which is placed in an electric drying oven at 130 °C until constant weight. The dried sample is cooled in a desiccator and weighted.

Ash content was determined through incineration in muffle according to ICC methods 104/1 (ICC, 1995). An aliquot of about 2-3 g is accurately weighted in a porcelain bowl which is placed in a muffle and incinerated at a temperature of 525 ± 10 °C. The residue is cooled in a desiccator and weighted.

Protein content (N × 5.70) was determined by combustion nitrogen analysis with Leco FP-528 (AOAC method 992.23) (AOAC, 2000).

The principle of the Dumas method is to convert nitrogen present in the sample into gaseous nitrogen oxides (NO_x) by complete combustion in a furnace maintained at 950 - 1.100 °C. The final product (NO_x) is then reduced to N_2 and measured using the thermal conductivity detector.

Cereal flour is weighed (200 mg) in tin foil (Leco tinfoil cups 502-186), using a foil holder (Leco 604-493), twisting the ends of the foil to form a teardrop-shaped pocket and then analyzed.

Total fat was analyzed by acidic hydrolysis (ICC method 136) (ICC, 1995) using hydrochloric acid in order to release lipids bound to proteins and sugar. After lipid extraction with diethyl and petroleum ether, the solvent is removed and the fat residue weighted.

Total arabinoxylans content was assessed according to Hashimoto et al. (1987).

Briefly, the sample is hydrolyzed at 100°C for 2.5 h with hydrochloric acid, after cooling, neutralization is affected by adding sodium carbonate. Fermentable sugars are removed by adding a suspension of fresh compressed yeast (*Saccharomyces cerevisiae*) to the sample that was incubated at 30°C until fermentation is complete.

The mixture containing pentosans is analyzed by the orcinol-HCl method, consisting in heating in boiling water for 30 minutes a solution composed of 2 mL of sample mixture, 1 mL of water, 3 mL of 0.1% ferric chloride in concentrated hydrochloric acid and 0.3 mL of 1% orcinol in absolute ethanol, cooling and determining absorbance at 670 nm.

Amylose content, total starch content (AOAC Method 996.11), resistant starch (AOAC method 2002.02), β-glucan (AOAC method 995.16), total dietary fiber (AOAC method 985.29) (AOAC, 2000), damaged starch (ICC method 164) (ICC, 1995) and α-Amylase activity level were determined using enzymatic assay kits (Megazyme Ltd, Ireland).

In the following paragraphs the principle of the methods is explained.

-For the determination of amylose content, samples are completely dispersed by heating in dimethyl sulphoxide (DMSO).

Lipids are removed by precipitating the starch in ethanol and recovering the precipitated starch. After dissolution of the precipitated sample in an acetate/salt solution, amylopectin is specifically precipitated by the addition of Con A and removed by centrifugation.

The amylose, in an aliquot of the supernatant, is enzymically hydrolyzed to D-glucose, which is analyzed using glucose oxidase/peroxidase reagent (GOPOD).

The total starch, in a separate aliquot of the acetate/salt solution, is similarly hydrolyzed to D-glucose and measured colorimetrically.

The concentration of amylose in the starch sample is estimated as the ratio of GOPOD absorbance at 510 nm of the supernatant of the Con A precipitated sample to that of the total starch sample.

-To evaluate total starch content two sets of enzymes are used. Thermostable α-amylase is used to hydrolyze starch into soluble, branched and unbranched maltodextrins, and then amyloglucosidase to hydrolyze maltodextrins to D-glucose which is quantitatively measured in a colorimetric reaction employing glucose oxidase/peroxidase.

Where necessary, resistant starch in the sample is pre-dissolved by stirring the sample with cold 1.7 M NaOH, followed by neutralization with sodium acetate buffer and hydrolysis with thermostable α-amylase.

-Resistant starch is determined incubating the samples in a shaking water bath with pancreatic α-amylase and amyloglucosidase for 16 h at 37°C, during which time non-resistant starch is solubilized and hydrolyzed to D-glucose by the combined action of the two enzymes.

Resistant starch is recovered as a pellet on centrifugation and dissolved in 2 M KOH. This solution is quantitatively hydrolyzed with amyloglucosidase to D-glucose, which is measured with glucose oxidase/peroxidase reagent.

-For β-glucan determination, samples are suspended and hydrated in a buffer solution of pH 6.5 and then incubated with purified lichenase enzyme and filtered. An aliquot of the filtrate is then hydrolyzed to completion with purified β-glucosidase.

The D-glucose produced is assayed using a glucose oxidase/peroxidase reagent.

-Total dietary fiber is determined on duplicate samples. Samples are incubated at ~ 100°C with heat stable α-amylase to give gelatinization, hydrolysis and depolymerization of starch; then samples are incubated at 60°C with protease (to solubilize and depolymerize proteins) and amyloglucosidase (to hydrolyze starch fragments to glucose); and finally samples are treated with four volumes of ethanol to precipitate soluble fiber and remove depolymerized protein and glucose (from starch). The residue is filtered; washed with 78% ethanol, 95% ethanol, and acetone; dried; and weighed. One duplicate is analyzed for protein and the other is incubated at 525°C to determine ash. The total dietary fiber (TDF) is the weight of the filtered and dried residue less the weight of the protein and ash.

-Damaged starch of flours is evaluated using a purified fungal α-amylase that gives a complete solubilization of damaged granules to maltosaccharides and α-limit dextrins with minimum breakdown of undamaged granules. This reaction is terminated on addition of dilute sulphuric acid, and aliquots are treated with excess levels of purified amyloglucosidase to give complete degradation of starch-derived dextrins to glucose that is specifically measured with a high purity glucose oxidase/peroxidase reagent mixture.

-For the determination of α-amylase activity level, an aliquot of a cereal flour extract is incubated with a substrate mixture (non-reducing-end blocked p-nitrophenyl maltoheptaoside in the presence of excess levels of a thermostable α-glucosidase), immediately α-amylase of the sample cleaves a bond within the blocked p-nitrophenyl

maltosaccharide substrate, the non-blocked reaction product is instantly cleaved to glucose and free p-nitrophenol by the excess quantities of thermostable α-glucosidase. The reaction is terminated with the addition of tri-sodium phosphate and the develop of phenolate color. The absorbance at 400 nm is measured and this relates directly to the level of α-amylase in the sample analyzed. Activity is expressed as Ceralpha Unit/g d.w. (hereinafter "U/g d.w.") according to the manufacturer's instructions.

4.2.8 Flours characteristics

The color was evaluated measuring L* a* b* values (CIE, 1986) with a Colorimeter mod. CR-300 (Konica Minolta Sensing Americas Inc, USA).

Particle size distribution of flours was evaluated by a mechanical sifter (Bühler, Uzwill, Switzerland). Flour (100 g) is placed on the topmost sieve of a nest of sieves of successively decreasing apertures (477 μm, 355 μm, 300 μm, 180 μm, 150 μm, 85 μm for flours obtained from hard-wheat mill, while 183 μm, 150 μm, 130 μm, 85 μm, 65 μm for flours obtained from soft-wheat mill), after 5 minutes of sifting, the mass of sample retained on each sieve is recorded.

Gluten quality was determined according to method AACC 38-12 (AACC, 2000) (Perten Instruments, Sweden), but a manual gluten extraction was performed by washing (with 2% NaCl solution) the dough obtained from 10 g flour and salt solution through a sieve, until starch is removed completely. Wet gluten is dried at 150°C for 4 minutes using a GLUTORK 2020 to obtain dry gluten.

To analyze the mixing behavior of the doughs, farinograph indices were assessed with Brabender farinograph (ICC method 115/1) (ICC, 1995).

In particular, the mixer of the instrument is filled with the flour, weighted on the basis of its moisture content, and mixed with a volume of water to give a maximum consistency of approximately 500 farinograph unit (FU). The maximum consistency of the dough is adjusted to a fixed value by altering the quantity of water added.
The dough is kept mixing for 20 min from the addition of water. During this period a curve is registered and it is used to calculate farinograph parameters (water absorption, dough development time, dough stability, degree of softening).

Alveograph properties of the dough were evaluated with Chopin-Alveograph (ICC method 121) (ICC, 1995). The dough from flour and 2.5% NaCl is normally prepared under standard conditions, but the method had to be adjusted for high amylose flours adding further 50 mL NaCl solution. Successively, the dough is formed into disc-shaped pieces and after a fixed resting period the pieces are inflated into bubbles. The pressure variation inside each bubble is recorded in a graph. These pressure variations describe the resistance to stretching and the extensibility of the dough tested. The length and the shape of the curve obtained from the extension of the bubble to the rupture are the criteria of the physical properties of a dough and hence of the baking characteristics of the flour. The following rheological parameters are obtained from the curve:

-P: dough tenacity (resistance);

-L: dough extensibility;

-G: dough elasticity;

-W: baking strength;

-P/L ratio: equilibrium of the flour comparing tenacity versus extensibility.

Gelatinization properties were assessed with ICC 126/1 (ICC, 1995) method using a Micro Visco-Amylo-Graph (MVA Brabender OHG, Duisburg, Germany). The method is based on the heating of a suspension of flour and distilled water from 30 to 95°C with a constant heating rate of 1.5° C/min, holding at 95°C for 20 min and cooling (1.5°C/min) until 50°C (1 min holding) within a rotating bowl.

Depending on the viscosity of the suspension, a measuring sensor reaching into the bowl is deflected. This deflection is measured as a function (viscosity) over time and recorded in a graph. The viscosity is expressed as Brabender Unit (BU). In the measurement diagram the following parameters are obtained:

- gelatinization temperature;
- peak viscosity (gelatinization maximum);
- breakdown (viscosity from peak viscosity to end of cooling period);
- setback (viscosity from end of cooling period to final holding period).

Falling number (FN) was determined according to ICC method 107/1 (ICC, 1995) (FALLING NUMBER mod. 1500, PERTEN).

The Falling Number Method uses the starch contained in the sample as a substrate. It is based on the rapid gelatinization of a suspension of flour in a boiling waterbath and the subsequent measurement of the liquefaction of the starch by alpha-amylase. In particular, the flour is weighted considering moisture content and put in the viscometer tube; successively, the sample is dispensed by adding 25 mL of distilled water.

Sample and water are mixed by vigorously shaking the tube to obtain a homogeneous suspension and then stirred putting the viscometer tube into the boiling water bath of the instrument. The stirrer is automatically released in its top position and is allowed to fall down.

The Falling Number is defined as the time in seconds required to stir and to allow the viscometer stirrer to fall.

4.2.9 Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics Base (Version 26). T-test was conducted on data of grain composition and on ash content of milling fractions compared to those obtained from HAW- MT1. ANOVA and Tukey HSD for post hoc comparison of means were performed on data obtained from chemical composition of flours. Significant difference was set for $p < 0.05$.

4.3 Results and discussion

4.3.1 Kernel characterization

In Table 4.1 results concerning grains quality attributes are reported. High amylose wheat showed a higher thousand kernel weight compared to wild type wheat, while test weight reached lower values in HAW (74.4 kg/hL) compared to normal genotype (76.4 kg/hL), coherently with previous studies (Schönhofen et al., 2017).

At a visual examination, kernels of high amylose wheat appear with a wrinkled surface and dull color. In addition, grain hardness was significantly increased in HAW compared to NSW (89 vs 62 HI) in line with previous observations (Botticella et al., 2018; Schönhofen et al., 2017).

HAW reported a higher level of ash, protein and lipid content than NSW coupled with a reduced total starch (TS) content as previously described (Schönhofen et al., 2017; Botticella et al., 2016, 2018; Slade et al., 2012; Hogg et al., 2017). Both samples

showed an arabinoxylan content of approximately 6% and a β-glucan content of about 1%.

The amylose content reached 58.1% in HAW, consequently, the amylose/amylopectin ratio was higher (1:0.7) compared to NSW (1:3).

HAW showed a higher resistant starch (RS) content (10% d.w.), representing 18% of total starch, while negligible levels were found in control wheat, coherently with previous works (Botticella et al., 2018; Regina et al., 2015). Despite resistant starch is considered a component of dietary fiber (Dai & Chau, 2017), the applied method for total dietary fiber (TDF) analysis does not quantitively measure resistant starch (McCleary, 2007; Van der Kaaij et al., 2009) so the resistant starch content was evaluated with the protocol described in sec. 4.2.7.

4.3.2 Milling characteristics of high amylose wheats (soft-wheat mill)

Table 4.2 reports yield and ash composition of fractions of both genotypes milled with tempering at 17% final moisture (HAW-MT1, NSW-MT1).

Overall, milling of high amylose wheat gave 49.5% flour yield compared to NSW which gave 65.7%. By contrast, yield of bran and shorts accounted for 48.8% of products obtained from milling of high amylose wheat, while 33% obtained for NSW.

Table 4.1. Quality and chemical composition of high amylose (HAW) and normal starch wheat (NSW).

	TKW (g)	Test weight (kg/hL)	HI	Ash (% d.w.)	Protein (% d.w.)*	Lipid (% d.w.)	AX-tot (% d.w.)	β-Glu (% d.w.)	TDF
HAW	43.5± 0.51a	74.4 ±0.14a	89±16a	2.13 ± 0.03a	11.7 ± 0.07a	4.3 ± 0.07a	6.2 ± 0.00a	1.0±0.03a	15.2 ± 0.35a
NSW	39.7± 0.90b	76.4 ±0.49b	62±15b	1.94 ± 0.03b	10.6 ± 0.02b	3.2 ± 0.08b	5.5 ± 0.24a	0.4±0.02b	12.3 ±0.80a

	TS (% d.w.)	AM (%)	AM/AP	RS (% d.w.)	RS (% TS)
HAW	61.0± 0.46a	58.1± 1.93a	1/0.7a	10.0 ± 1.37a	17.7±2.13a
NSW	65.5± 0.12b	23.4± 0.33b	1/3.2b	0.3 ± 0.21b	0.5±0.32b

Mean values ± sd. Different letters in a column indicate statistically significant differences ($p < 0.05$, T-test). *N × 5.70; TKW, thousand kernel weight; HI, hardness index; AX, arabinoxylan; β-Glu, beta-glucan; TDF, total dietary fiber; TS, total starch; AM, amylose; AP, amylopectin; RS, resistant starch.

HAW flour obtained from breaking rolls reached an ash content of 1.24% d.w., higher compared with the 0.52% d.w. value in NSW, while HAW flours obtained with reduction rolls gave 0.79% d.w. of ash instead of 0.47% d.w. of control wheat. In turn, ash content decreased in bran and shorts compared to NSW, indicating, together with the higher yield of these fractions, that part of the flour that should be conveyed in all fractions from B1 to C3 is collected as a by-product.

The results suggest that kernels with modified starch composition behaves differently in a conventional milling, with lower flour yield and difficulties in a complete separation of starchy endosperm from the pericarp, in accordance with other authors (Hogg & Giroux, 2019; Schönhofen et al., 2017). This could be due to the lower starch content and thicker bran layers, or there could have been more endosperm adhering to bran which resulted in lower flour yield.

Results can also be linked to the phenotypic features of the kernel, appearing with a rougher surface which may limit abrasion of rolls on the grain.

Moreover, the higher accumulation of non-starch polysaccharides might have influenced tempering (Xu et al., 2018).

4.3.3 Effects of reduced tempering on HAW milling performance

Previous authors (Hogg & Giroux, 2019) improved milling performance of high amylose wheat by reducing tempering, thus in this study HAW moisture was decreased at 13% and milling was performed with soft-wheat mill (HAW-MT2).

Flour yield from breaking and reduction rolls improved (+6.6%) with lower amount of bran and shorts (- 8.2%) (Table 4.2). Ash content in the whole flour was overall comparable to the 17% tempering (0.88% d.w.), indicating that reduced water content only allowed a redistribution of pericarp fractions in B1–C3 flours.

4.3.4 Effects of debranning on HAW milling performance

To reduce ash content in flours, debranning was applied prior to conventional milling to increase efficacy of bran-endosperm separation.

The most suitable debranning rate was evaluated using a laboratory debranning removing 5–30% outer layers.

As expected debranning caused a reduction of ash content in both high amylose wheat and normal wheat, however, the sharpest reduction of ash content appeared between 5 and 10% debranning, thus in this study high amylose wheat was treated at 6% before milling to evaluate the impact on flour yield and ash content.

An increase of flour yield was observed (Table 4.2 HAW-MT3) compared to both previous diagrams (53% at tempering of 13% and 49.5% at final tempering of 17%).

On the other hand, ash content increased in breaking fractions compared to previous milling and dropped in reductions, balancing the total ash content in the flour (including all fractions B1–C3) that was comparable to previous milling diagrams (0.88% d.w.); at the same time, ash content was significantly lower in bran and shorts ($p < 0.05$) compared to conventional milling.

However, higher flour yields were recorded in C1–C3 flour, with an improvement of refining rate (ash content = 0.75% d.w.) compared to previous trials (0.80% d.w. HAW- MT2, 0.79% d.w. HAW-MT1).

Results indicate that debranning mainly affected the distribution of pericarp in the fractions but is a strategy that can be successfully applied to high amylose wheat to improve yield of total flour while obtaining more refined reduction flour mix.

Table 4.2. Yield and ash content of fractions obtained from the different milling trials.

Fractions	**HAW-MT1**		**NSW-MT1**		**HAW-MT2**		**HAW-MT3**		**HAW-MT4**	
	Yield (%)	**Ash (% d.w.)**	**Yield (%)**	**Ash (% d.w.)**	**Yield (%)**	**Ash (% d.w.)**	**Yield (%)**	**Ash (% d.w.)**	Yield (%)	Ash (% d.w.)
B1	6.5	1.07±0.01	12.7	0.43±0.02*	7.1	1.01±0.02	6.6	1.50±0.00*	2.4	1.27±0.02
B2	2.5	1.46±0.13	3.4	0.65±0.01*	2.7	1.47±0.03	2.0	1.67±0.02	11.7	0.69±0.02
B3	1.3	1.70±0.08	1.8	0.89±0.00*	1.2	1.69±0.02	0.8	1.53±0.02	3.1	0.75±0.04
Breaking rolls[a]	*10.2*	*1.24*	*17.8*	*0.52*	*11.0*	*1.19*	*9.4*	*1.54*	**17.2**	**0.78**
C1	17.0	0.92±0.07	29.0	0.44±0.25*	19.7	0.84±0.05	22.3	0.73±0.02*	9.0	0.70±0.01
C2	12.6	0.74±0.06	13.0	0.39±0.23*	13.9	0.68±0.01	15.2	0.66±0.05	3.2	0.85±0.02
C3	9.6	0.63±0.03	5.8	0.85±0.01*	8.4	0.92±0.00*	8.4	0.95±0.00	2.8	1.32±0.01
Reduction rolls[a]	*39.2*	*0.79*	*47.8*	*0.47*	*42*	*0.80*	*45.9*	*0.75*	**15.0**	**0.85**
Refined semolina	-	-	-	-	-	-	-	-	**34.1**	**0.70±0.02**
Total flour[a]	**49.5**	**0.88**	**65.7**	**0.49**	**53.0**	**0.88**	**54.4**	**0.88**	**66.3**	**0.75**
Bran	7.5	4.44±0.08	10.1	4.80±0.03*	4.1	4.28±0.00	1.8	3.81±0.08*	17.3	4.14±0.00
Shorts	41.3	2.57±0.03	22.9	3.03±0.07*	41.0	2.82±0.01*	32.5	3.22±0.02*	8.3	4.57±0.02
Milling by-products[a]	**48.8**	**2.86**	**33.0**	**3.58**	**45.1**	**2.95**	**33.7**	**3.25**	**25.6**	**4.28**
Debranning by-product	**-**	**-**	**-**	**-**	**-**	**-**	**6.0**	**6.68±0.03**	**6.0**	6.68±0.03

[a] Average values were calculated according to the yield of each fraction. HAW-MT1, high amylose wheat milled at 17% tempering. NSW-MT1 normal soft-wheat milled at 17% tempering. HAW-MT2, high amylose wheat milled at 13% tempering. HAW-MT3 high amylose wheat debranned at 6% and milled. HAW-MT4, high amylose grain debranned at 6%, tempered (14% moisture) and milled with hard-wheat mill.

Effects of debranning and milling with a hard-wheat mill on HAW milling performance

In this study, HAW exhibited a grain hardness similar to durum wheat, and as a consequence, a milling protocol with debranning (6%) and a hard-wheat milling diagram was tested on this sample. Semolina type flour yield reached 34%, while flour obtained from breaking and reduction rolls reached 32.2% (Table 4.2). In total 66.3% flour was obtained, which outdid previous recorded yields.

Overall, ash content decreased in fractions when compared with all the previous milling diagrams.

A better separation was obtained from break rolls, with the flour reaching the lowest value of ash (0.78% d.w.).

In addition, the flour obtained from sizing rolls had 0.85% d.w. ash while semolina had 0.70% d.w. ash content. In turn, by-products resulted enriched in ash compared to previous trials.

Results indicate that milling with hard-wheat mill gave the best performance in bran-endosperm separation improving flour yield and resulting in flour products that can be employed for the production of bakery foods, and products similar to semolina that can be conveniently used to produce products like pasta.

4.3.5 Flours characteristics

In Figure 4.4 flours particle size distribution of the selected flours is summed up in a graph, while in Table 4.3 flours quality traits and composition are reported. HAF showed a larger proportion of particle size >130 μm compared to NSF, while for HAF(B2C1) it is mainly distributed between 85 and 355 μm; on the other hand, high amylose semolina has almost 80% of particles greater than 300 μm (Figure 4.4 B).

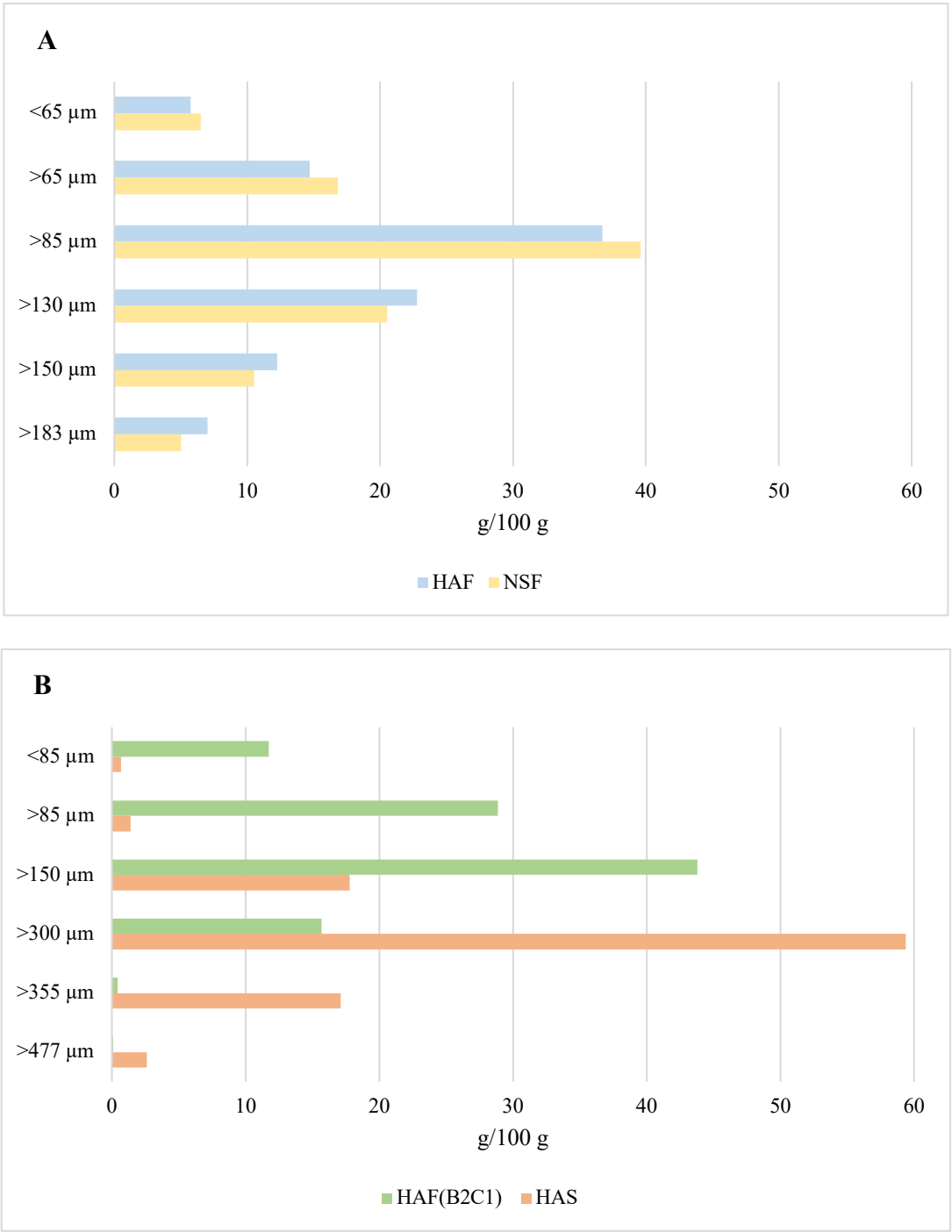

Figure 4.4. Average particle size distribution of flours. (A) HAF (C1-C3 fractions, high amylose grain debranned at 6% and milled with a soft-wheat mill) and NSF (fractions B1-C3 from normal grain milling) (B) HAF(B2C1) (high amylose flour - B2+C1- grain debranned at 6% and milled with a hard-wheat mill), HAS (high amylose semolina).

The same milling protocol applied to HAW and NSW generated a higher average proportion (8.6% total starch (TS)) of damaged starch in high amylose flours (Table 4.3) resulting from the harder endosperm texture (Wang et al., 2020), while in high amylose semolina this parameter was more than halved due to coarse particle size and lower grain pulverization (Wang et al., 2020).

The decreased refining rate of high amylose flours reduced L* values compared to NSF, while increasing a* and b* indices (Hogg et al., 2017). Flours obtained from hard-wheat mill exhibited higher L* values ($p < 0.05$) than HAF because of the greater particle size that enhances light reflectance (Manthey & Hareland, 2001).

Ash content in high amylose flours varied between 0.67% d.w. and 0.77% d.w. resulting higher when compared with normal starch flour.

As expected, TS content increased in NSF (80.9% d.w.) and in HAF (B2C1) (79.8% d.w.) which also gave the lowest ash content.

Total dietary fiber (TDF) in high amylose flours was more than doubled compared to normal starch flour (on average 6.8% d.w. compared to 2.5% d.w.), which can be attributed to the presence of bran parts and eventually higher resistant starch fraction.

Concerning starch composition, amylose content and AM/AP displayed comparable levels as the grains. RS content varied between 16.9% and 21.7% d.w. in high amylose flours, and in the range of 24.6–28.9% of the total starch content.

On average, flours obtained from hard-wheat milling showed the highest level of RS and this result can be explained by coarse granulometry (Roman & Martinez, 2019), since higher particle size distribution decreases the rate of starch digestion in relation to the amount of broken cells and α-amylase diffusion through the fragments (Al-Rabadi et al., 2009).

Table 4.3. Flours color (CIE index) and composition.

	L*	a*	b*	Ash (% d.w.)	Protein* (% d.w.)	Fat (% d.w.)	TDF (% d.w.)
HAF	66.7±0.21a	-0.7 ± 0.03a	+8.9 ± 0.10a	0.77 ± 0.000a	12.3 ± 0.07a	3.4±0.09a	6.4 ± 0.18a
HAF (B2C1)	61.8±0.19b	+0.0 ± 0.06b	+10.7±0.07b	0.67 ± 0.000b	11.0 ± 0.04b	2.1±0.00b	5.8 ± 0.05a
HAS	61.8±0.63b	-0.8 ± 0.16a	+11.7±0.19c	0.71 ± 0.017b	10.8 ± 0.12b	2.1±0.05b	8.2 ± 0.43b
NSF	68.9 ± 0.38c	-1.6 ± 0.02c	+7.6 ± 0.10d	0.48 ± 0.01c	8.6 ± 0.04c	2.3±0.11b	2.5 ± 0.05c

	TS (% d.w.)	AM (%)	AM/AP	RS (% d.w.)	RS (% TS)	DS (% TS)
HAF	68.4 ± 4.86a	54.0 ± 0.22a	1/0.8a	16.9 ± 0.87a	24.6 ± 1.28a	9.0 ± 0.94a
HAF (B2C1)	79.8 ± 2.00b	54.9 ± 0.07a	1/0.8a	21.7 ± 0.29b	27.2 ± 0.37ab	8.3 ± 0.85a
HAS	73.1 ± 0.50ab	54.3 ± 1.70a	1/0.8a	21.1 ± 0.23b	28.9 ± 0.32b	3.0 ± 0.49b
NSF	80.9 ± 0.50b	26.3 ± 0.87b	1/ 2.9b	0.4 ± 0.08c	0.5 ± 0.10c	7.2 ± 0.49a

Mean values ± sd. Different letters in a column indicate statistically significant difference ($p < 0.05$). *N × 5.70; TDF, total dietary fiber; TS, total starch; AM, amylose; AP, amylopectin; RS, resistant starch; DS, damaged starch.
HAF (C1–C3 fractions, high amylose grain debranned at 6% and milled with a soft-wheat mill); HAF(B2C1) (high amylose flour – B2+C1- grain debranned at 6% and milled with a hard-wheat mill); HAS (high amylose semolina); NSF (fractions B1–C3 from normal grain milling).

In addition, the lower level of damaged starch (DS) (Table 4.3) decreased the rate of starch hydrolysis, as this fraction is more susceptible to enzymatic action (Wang et al., 2020).

The results obtained in this study highlight that high amylose flours can serve as raw material for the production of functional foods enriched in RS also in blends with normal flours. Specifically, according to EU Reg. 432/2012 the derived products, with at least 14% substitution of total starch with resistant starch, might bear a health *claim* regarding the reduction of postprandial glycemia. At the same time, RS is part of dietary fiber, and thus contributes to increase the proportion of total undigestible carbohydrates, possibly reaching the target established by nutritional *claims* listed in EC Reg. 1924/2006.

4.3.6 Dough quality

In Table 4.4 dough properties of the different flours/semolina are reported. The official method for gluten quality determination AACC 38-12 was not applicable to high amylose flours, because of difficulties in modulating the operative conditions of the apparatus. Gluten index (GI), obtained performing a manual gluten extraction, varied between 87 and 93 in high amylose flour with a gluten content of 7.7% d.w. on average. Overall, gluten quality of high amylose flours can be considered acceptable, but the analytical protocol may require adaptation due to modified starch composition that can impact dough properties.

Farinograph analysis showed an increase in water absorption in high amylose flours which resulted 73.3% compared to 51.5% in conventional flour (NSF). These findings are in line with the work of Jaksics et al. (2020) in which this parameter significantly marked waxy (62–69%) and high amylose (80–85%) wheats groups; these results can

be attributed to the increase of RS (Barros et al., 2018) and other undigestible polysaccharides originating from bran contamination (Hung et al., 2007). The lower water absorption of HAS compared to the other high amylose flours could instead be attributed to the reduced DS. Dough development time and stability resulted increased in high amylose flours with lower degree of softening (DSf) at both 10 (3–46 BU) and 12 min (29–76 BU), compared to normal starch flour (111 and 120 BU). However, these data do not agree with previous investigations on high amylose flours and the role of RS on dough rheology (Barros et al., 2018; Schönhofen et al., 2017). The dough stability is usually related to the gluten strength, which in this study resulted comparable between high amylose flours and normal flour, and taken together, the data indicate a higher resistance of high amylose dough to processing specifically related to semolina-like flour.

Chopin alveograph, that works at constant hydration conditions, was not applicable to high amylose flour, requiring additional NaCl solution for the dough formation. Alveograph indices obtained at adapted hydration according to the farinograph analysis, revealed average W values slightly lower than normal flour (135 *10 $^{-4}$ J) with semolina showing the lowest value. On the other hand, higher P/L values (1.68–4.27) were obtained for high amylose flours compared to control flour (1.03). The same trend was detected in other studies for high amylose semolina (Botticella et al., 2016) and dough with partial substitution with RS (Barros et al., 2018). Alveograph results indicate that high amylose flours could reduce dough expansion in fermented products.

Regarding micro visco-amylo-graph indices, increased gelatinization temperature was detected in high amylose flour, spanning from 88.6 °C to 95.4 °C, compared to 67.1 °C in NSF. Viscosity of high amylose wheat did not change significantly over time,

and this trend was already described by Jaksics et al. (2020). Peak viscosity was on average 35 BU in high amylose flours compared to 188 BU in NSF, coherently with previous findings (Botticella et al., 2018; Jaksics et al., 2020; Schönhofen et al., 2017) and attributable to the higher amylose proportion that limited water absorption and swelling preventing disruption of granule structure (Sasaki, 2005), thus affecting starch gelatinization dynamic in baking. Moreover, the higher gelatinization temperature found in semolina, compared to other high amylose flour, can be linked to reduced starch damage (Tosi et al., 2018). Decreased breakdown in high amylose flours support a higher resistance of starch granules to shear stress and disintegration at high temperature (Shevkani et al., 2016). Setback values usually indicate the magnitude of starch retrogradation (Wang et al., 2015) and in this study, high amylose flours presented values of 20–29 compared to 146 found in normal starch flour.

The modification of starch paste viscosity may affect the falling number (FN) measurement. In this study, normal starch flour showed a FN of 473 s while the modified genotype gave FN of 62 s (Table 4.4) for all the samples considered, a value more likely to be found in sprouted grains (Olaerts et al., 2017). However direct evaluation of enzymatic activity showed no difference in α-amylase level in high amylose grains compared to normal wheat grains (0.21 U/g d.w. − 0.17 U/g d.w.) as well as in flours, despite the higher value in high amylose flours may be attributed to the inclusion of the outer layers of the grain. Previous authors working on waxy wheat also reported low FN values, which were not correlated with α-amylase activity (Abdel-Aal et al., 2002) because of the altered AM/AP ratio. In the light of the recorded performances, we suggest that α-amylase activity should be verified with an enzymatic evaluation since the altered pasting profile of high amylose flours affected the FN measurement procedure.

Table 4.4. Gluten index and dry gluten content (manual extraction), average farinograph, alveograph and micro-visco-amylograph indices, FN and α-amylase activity of flours.

	GI	Dry gluten (% d.w.)	WA (%)	DDT (min)	DSt (min)	DSf (BU) (10 min)	DSf (BU) (12 min)	W (10^{-4} J)	P/L	GT (°C)	Peak viscosity (BU)	Breakdown (BU)	Setback (BU)	FN (s)	α- Amylase activity (U/g d.w.)
HAF	87	9.3 ± 0.25	76.0	2.6	4.7	46	76	169	1.68	88.6	40	1	29	62	0.19± 0.03
HAF (B2C1)	93	6.1 ± 0.08	73.3	3.0	6.4	32	56	141	2.50	93.0	33	2	24	62	0.11±0.01
HAS	93	7.5 ±0.06	70.6	8.1	12.5	3	29	96	4.27	95.4	32	4	20	62	0.12±0.00
NSF	92	7.5 ± 0.12	51.5	1.1	1.2	111	120	143	1.03	67.1	188	22	146	473	0.11 ± 0.01

GI, gluten index; WA, water absorption; DDT, dough-development time; DSt, dough stability; DSf, degree of softening; GT, gelatinization temperature; FN, falling number.
HAF (C1–C3 fractions, high amylose grain debranned at 6% and milled with a soft-wheat mill); HAF(B2C1) (high amylose flour – B2+C1- grain debranned at 6% and milled with a hard-wheat mill); HAS (high amylose semolina); NSF (fractions B1–C3 from normal grain milling).

4.4 Conclusions

Debranning of high amylose wheat and the further application of a hard-wheat milling diagram increased yield and improved milling performance, giving a semolina-type flour that can be conveniently employed to produce semolina-based products (pasta, couscous).

High amylose flours showed increased RS content compared to normal wheat flour making them useful to formulate innovative products in view of health and nutritional *claims* (Regulations EU 432/2012 and EC 1924/2006).

Moreover, the results showed that official methods for rheological analysis might need adaptation due to the AM/AP ratio that affects dough properties (water absorption, viscosity).

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Chapter 5: HIGH AMYLOSE BREAD WHEAT SEMOLINA AND ITS EFFECTS ON COOKING QUALITY AND NUTRITIONAL PROPERTIES OF PASTA

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High amylose bread wheat semolina and its effects on cooking quality and nutritional properties of pasta.
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Abstract

Pasta samples were produced by replacing durum wheat semolina with high amylose bread wheat semolina in proportions of 30%, 50%, 70%. High amylose pasta composition, nutritional and cooking performances were evaluated in comparison with semolina control pasta. Resistant starch content in uncooked pasta samples varied from 4.9% of total starch in high amylose pasta with 30% substitution of semolina, to 15.3% of total starch in 100% high amylose semolina pasta, achieving the levels established for the health *claim* (Reg. EU 432/2012) and an attenuation of post-prandial glycemia. High amylose flour impacted on cooking performances and textural properties. The resistant starch content however, was still present in a considerable amount in cooked pasta, a property that positively affected the expected glycemic index, causing a reduction of starch digestion rate in all samples with high amylose flour substitution compared to control. In particular, the expected glycemic index lowered from 53.8 in control pasta (durum wheat semolina) to 48 in 100% high amylose pasta (bread wheat semolina). The inclusion of high amylose flour in proportion of 70% combines the best cooking and nutritional properties, but it is expectable a further improvement of pasta quality with the adoption of tailored processing protocols.

Key words: *cooking quality, glycemic index, resistant starch, starch hydrolysis, texture.*

5.1 Introduction

Pasta is a staple food consumed all over the world, for its simple handle use, affordability and source of complex carbohydrates.

The replacement of digestible carbohydrates with undigestible ones positively impacts the glycemic load after a meal, nevertheless, in starchy foods, the type of starch and its rate of digestion (rapidly digestible, slowly digestible and resistant), as well as food composition and preparation are important determinants of the metabolic responses (Aller et al., 2011; Bornet et al., 1997; Frost & Dornhorst, 2000).

Starch is the main component in wheat and consists of glucose polymers, amylose, with a linear structure of α-1,4 linkages, and amylopectin, highly branched with both α-1,4 and α-1,6 linkages (Shen et al., 2021). These macropolymers are organized with a usual ratio of 1:3 in wheat (Tester et al., 2004) althought the molecular characteristics vary in other cereal grains such as maize (Lubowa et al., 2021) or rice (Ma et al., 2020; Puhin et al., 2021).

Over the last years, modified wheat genotypes with a higher amylose proportion have been developed, since evidences showed a higher resistant starch fraction as a result of increased amylose proportion (Regina et al., 2006; Sestili et al., 2010).

Resistant starch is defined as the portion of starch that reaches the gut undigested and is fermented by the microbiota (Birt et al., 2013; Petchoo et al., 2020). The resistance to digestion is attributable to mechanisms like reduced granule disruption, formation of amylose-lipids complex, reduced swelling, higher retrogradation rate (Bird & Regina, 2018).

The replacement of digestible starch with resistant starch lowers the glycemic index of derived foods (Hallström et al., 2011; Sissons et al., 2020), an effect supported by

the health *claim* enlisted in Reg. EU 432/2012 related to the reduced postprandial glycemia with a minimal proportion of 14% resistant starch over total starch.

In addition, resistant starch is included in the definition of dietary fiber, making it a valid component to enhance the undigestible polysaccharides content of the final products, in view of nutritional *claims* (Reg. EC 1924/2006).

At this regard the resistant starch arrives intact in the colon, where it is fermented by the gut microbiota, resulting mainly in the production of beneficial short-chain fatty acids (SCFAs) (acetate, propionate, and butyrate). SCFAs, overall butyrate, play an important in maintaining gut health by reducing inflammation and the risk of colorectal cancer (Nugent, 2005; Bird et al., 2013). Recently, some studies demonstrated an interaction between resistant starch and microbiome. In detail, foods rich in resistant starch restore gut microbiome homeostasis through the selection of butyrate-producing bacterial communities (Dobranowski & Stintzi, 2021). In bread wheats the increase in amylose content causes a harder grain texture (> 80 HI) (Botticella et al., 2018; Hogg et al., 2017). For this, in our previous study (De Arcangelis et al., 2021) we explored the opportunity to apply the hard-wheat milling diagram also to high amylose bread wheat to obtain a semolina type product (particle size > 300µm). In the same study the resulting semolina-type flour was characterized and identified as a potential raw material for products that are normally obtained with durum wheat semolina (pasta and couscous).

Processing methods such as cooking, deeply affect starch constituents and, consequently, properties of starchy cooked foods. When starch is heated in sufficient water an irreversible phase transition phenomenon occurs, known as gelatinization, with subsequent hydration, swelling and disruption of the crystalline structure of starch

(Shevkani et al., 2017). Upon cooling, retrogradation takes place where amylose and amylopectin reassociate forming an ordered structure (Wang et al., 2015).

These events may impact glycemic index (GI), as gelatinized starch is more susceptible to enzyme action, a property which largely depends on the extent of granule disruption during heating (Wang et al., 2015). Conversely, cooling of gelatinized starch lowers the GI of products as a results of starch retrogradation (Frost & Dornhorst, 2000). To the best of our knowledge, few studies (Hazard et al., 2015; Hogg et al., 2015; Sissons et al., 2020) have been conducted on the effects of high amylose wheat on pasta products, so, the objective of this work is to elucidate the influence of the inclusion of high amylose bread wheat on textural and nutritional properties of cooked pasta.

5.2 Materials and methods

5.2.1 Samples

High amylose bread wheat cv. Cadenza (amylose content= 58.2% over total starch, Hardness Index=89) was provided by University of Tuscia (Viterbo, Italy). Given the harder kernel texture of high amylose wheat compared to a conventional bread wheat, grains were grinded using a hard-wheat mill in combination with debranning, allowing the implementation of milling performance according to a previous study (De Arcangelis et al., 2021). Grain was debranned using a debranner (NAMAD, Roma, Italia) at 6%. Prior to milling, water was added to the debranned kernels to reach 14% (*w/w*) moisture and left for twelve hours at room temperature. Milling was performed using the pilot plant "MLU 202" (Bühler, Uzwill, Switzerland) following AACC International methods 26-10A and 26.41 (AACC 2000). Sifted semolina-type flour

(hereinafter as high amylose semolina) was used as raw material for pasta production. Commercial durum wheat semolina was purchased in a local market and used as control.

5.2.2 Particle size distribution and gluten index

The methods for the evaluation of particle size distribution and gluten quality of semolina are carried out according to De Arcangelis et al. (2021).

5.2.3 Pasta preparation

Five formulations of pasta (tagliatelle shape) were prepared using high amylose semolina-type flour and durum wheat semolina (Table 5.1). Flours were mixed with the required water content, in respect of the higher water absorption of high amylose flour (De Arcangelis et al., 2021). Mixing and dough extrusion were performed using a pasta maker "La perfetta medium" (La Prestigiosa, Villaverla (VI), Italy). To study the sole effect of high amylose flour, pasta underwent a mild heat treatment to desiccate using a static dryer (Namad Impianti, Rome, Italy) at 30°C for 24h (Figure 5.1) Aliquots of blends and uncooked pasta samples milled with a refrigerated mill (IKA A10, Staufen) were kept for analysis.

Table 5.1. Pasta formulations

SAMPLE	DURUM WHEAT SEMOLINA	HIGH AMYLOSE SEMOLINA	WATER
	(%)	(%)	(%)
P-B30-HA-S	70	30	35.0
P-B50-HA-S	50	50	37.5
P-B70-HA-S	30	70	40.0
P-HA-S	0	100	47.5
P-S	100	0	28.0

P-B30-HA-S, pasta 30% high amylose semolina and 70% semolina; P-B50-HA-S, pasta 50% high amylose semolina and 50% semolina; P-B70-HA-S, pasta 70% high amylose semolina and 30% semolina; P-HA-S, pasta 100% high amylose semolina; P-S, pasta 100% semolina

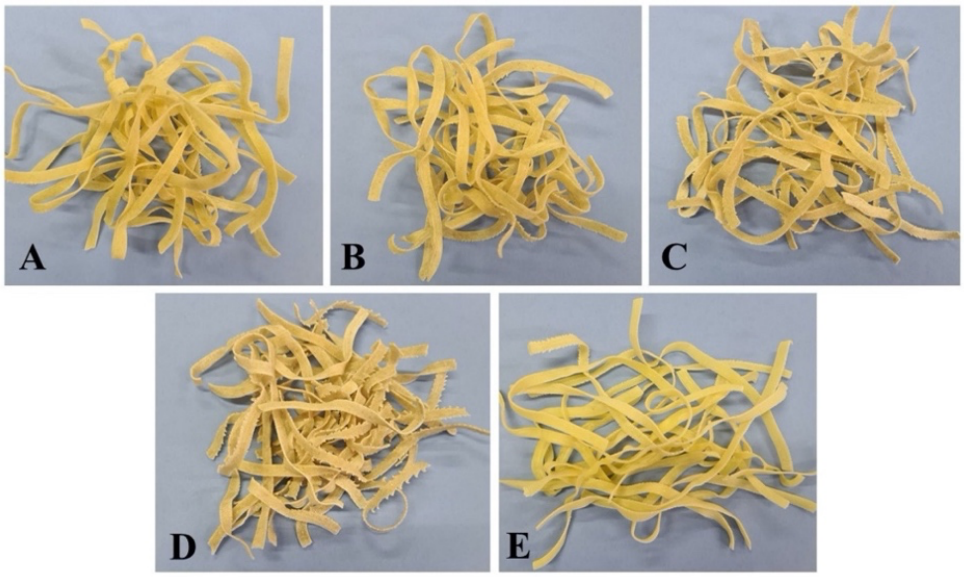

Figure 5.1. Pasta samples. A= P-B30-HA-S pasta 30% high amylose semolina and 70% semolina; B=P-B50-HA-S pasta 50% high amylose semolina and 50% semolina; C=P-B70-HA-S pasta 70% high amylose semolina and 30% semolina; D= P-HA-S pasta 100% high amylose semolina; E= P-S pasta 100% semolina.

5.2.4 Cooking conditions

Pasta was cooked in boiling tap water (in ratio 1/10 *w/v*) until the optimum cooking time (OCT) was reached as defined as the time necessary to obtain complete gelatinization of starch, shown by the disappearance of the white central core of the strand by squeezing the pasta between two plates of glass (ISO 2016).

5.2.5 Chemical analysis

The determination of moisture, protein, ash, total starch, amylose, damaged starch, resistant starch and total dietary fiber was carried out according to procedures reported by De Arcangelis et al. (2021).

5.2.6 Color evaluation

Color was determined on uncooked pasta using a reflectance colorimeter (CR200 Chromameter, Minolta, Japan) measuring b* index (positive value is yellowness and negative value is blueness). For the evaluation, tagliatelle samples were arranged close together in rows to minimize air space.

5.2.7 In vitro starch digestibility and expected glycemic index

In vitro kinetic starch digestion of cooked pasta was evaluated according to Romano et al. (2016) with minor modifications. All samples were analyzed "as eaten" and were freshly prepared before *in vitro* analyses. Cooked pasta was drained for 1 min and cut in small size to simulate mastication. Each sample (100 mg) was incubated with 4 mL of 100 mM sodium acetate buffer (pH 6) containing 0.4 mg of pancreatic α-amylase

(3000 U/g Megazyme) and 40 µL of amyloglucosidase (300 U/mL Megazyme) in a shaking water bath (200 strokes/min, horizontal agitation) at 37°C for 0, 30, 60, 120, 180 min. The reaction was stopped by adding absolute ethanol (4 mL), then the samples were centrifugated at 3500 rpm for 10 min and the supernatants recovered. The centrifugation was repeated twice more with 8 mL of aqueous ethanol (50% *v/v*). All the supernatants were pooled together and diluted to 100 mL with 100 mM sodium acetate buffer (pH 4.5). Aliquots of 0.1 mL (in duplicates) were incubated with 10 µL of amyloglucosidase (300 U/mL) for 20 min at 50°C. Finally, the amount of glucose released was measured spectrophotometrically using glucose oxidase/peroxidase (GOPOD) kit. Values (expressed as % hydrolyzed starch/100 g of cooked sample) were plotted on a graph vs. time, and the area under the hydrolysis curve (AUHC; 0–180 min) was measured by using the trapezoid rule. A starch hydrolysis index (HI) value was calculated as the ratio between the area under the hydrolysis curve of each sample and the corresponding area of white wheat bread expressed as a percentage. From HI, the estimated glycemic index (eGI) was calculated using the following formula: eGI=39.71+ 0.549HI (Goñi et al., 1997).

5.2.8 Pasta cooking quality

-Firmness, liveness and starch release of cooked pasta were evaluated according to ISO 7304-1 (ISO, 2016). A scale ranging from 10 to 100 was used. The final values of the cooking quality obtained from the mean of the scores given by all experts is then correlated with a description. Pasta with a total score below or equal to 40 was of poor or mediocre quality; above 40 but below or equal to 50, the quality was not completely satisfactory; above 50 but below or equal to 70, it was fair; above 70 but below or equal to 80, it was good; above 80, it was excellent.

-Cooking loss was evaluated by determining the amount of the solids lost in cooking water according to Marti et al. (2013). Briefly, pasta was cooked in natural spring water, the cooking water was recovered and diluted to the initial volume. An aliquot of 25 mL was dried until constant weight at 105°C. The residue was weighed and reported as a percentage of the original weight of pasta sample.

-Water absorption index (WAI) and swelling index (SI) were calculated according to Foschia et al. (2015). Cooked pasta, after draining and cooling, was weighed and WAI was calculated using the following equation:

$$WAI = \frac{\text{weight of cooked pasta} - \text{weight of dry pasta}}{\text{weight of dry pasta}} * 100$$

SI was determined by drying cooked pasta to constant weight at 105°C. It was expressed as:

$$SI = \frac{\text{weight of cooked pasta} - \text{weight of pasta after drying}}{\text{weight of pasta after drying}}$$

5.2.9 Texture analysis

The textural characteristics of cooked pasta were measured by Texture Analyser (TA.XT2, Stable Micro System, UK) equipped with a 5 kg load cell according to AACC Approved Method 66–50. The cutting test was conducted through a blade probe (HDP/LKBF) (setting pre-speed 1.0 mm/s, test speed 1.0 mm/s, post-speed 1.0 mm/s, distance 100.0%). Each cooking test was carried out in duplicate, and the numerical results are averages of at least 5 independent replicates. Hardness is considered as the maximum cutting force (g) while firmness is considered as the cutting work (g s) required to shear three strands of cooked tagliatelle (Larrosa et al., 2016; Szczesniak, 2002).

5.2.10 Statistical analysis

Statistical analysis was performed using R software (version 3.6.3). Data were subjected to analysis of variance (ANOVA) followed by Tukey's post-hoc test. Results with $p < 0.05$ indicate statistically significant difference. The relationship between parameters was measured using the linear correlation coefficient (r).

5.3 Results and discussion

5.3.1 Characterization of raw materials

Table 5.2 reports the gluten quality and composition of high amylose semolina (HA-S) and durum wheat semolina used as a control (S). A manual gluten extraction was performed, in tandem with the properties of high amylose doughs as reported by De Arcangelis et al. (2021).

Table 5.2. Gluten index, chemical composition (ash, protein and total dietary fiber) and particle size distribution of high amylose bread wheat semolina and durum wheat semolina

	GI	ASH	PROTEIN N×5.70	TDF	>477 µm	>355 µm	>300 µm	>183 µm	>85 µm	<85 µm
		% dw	% dw	% dw	%	%	%	%	%	%
HA-S	76	0.91±0.01a	10.4±0.12b	8.6±0.23a	2.8	16.3	58.5	19.3	1.8	0.7
S	73	0.81±0.00b	14.2±0.04a	2.6±0.33b	7.6	19.3	39.8	17.0	10.8	4.7

HA-S, high amylose semolina; S, durum wheat semolina; GI, gluten index; TDF, total dietary fiber

The gluten index was comparable between the samples, with a higher protein content in control semolina (14.2% d.w.) compared to high amylose semolina (10.4% d.w.). HA-S presented a higher ash content than the control semolina, due to the difficulty in a complete endosperm-bran separation during milling, as previously reported (De

Arcangelis et al., 2021). The total dietary fiber content reached 8.6% in high amylose semolina compared to 2.6% in the control semolina, related to an increased resistant starch proportion.

A more uniform particle size distribution (Table 5.2) was recorded for high amylose semolina with almost 60% of the material ranging between 300 and 355 μm. Approximately 78% of particles had a dimension > 300 μm in HA-S versus 67% in S. In commercial semolina a largest proportion of particles (27%) had a dimension > 355 μm compared to high amylose sample (19%). These results illustrate that milling a high amylose bread wheat with a hard wheat mill gives a product which has a coarse particle size, very similar to a durum wheat semolina.

In table 5.3 the starch composition of blends and semolina is reported.

The total starch content is significantly different between HA-S and S (69.1% and 78.6%), affecting the level of total starch (TS) in blends which spanned from 69.5% d.w. to 76.2% d.w. The amylose fraction in HA-S was comparable with the starting grain (58.2%) and proportional in the corresponding blends.

The resistant starch reached almost 30% of the total starch content in HA-S compared to < 1% in S. As a consequence, in blends with a higher supplementation of HA-S there was an increase in RS levels, reaching 21.2% TS in blend with 70% of HA-S (B70-HA-S). Damaged starch in S was almost doubled compared to HA-S (5.4% versus 2.8%), thus the level in blends progressively reduced with the proportion of high amylose semolina.

Table 5.3. Starch content and composition of high-amylose blends and semolina

	TS	RS	RS	DS	AMYLOSE
	%dw	%dw	% TS	%TS	% TS
B30-HA-S	76.2±3.84a	7.3±0.12d	9.5±0.15 d	4.5±0.27b	40.2±2.15d
B50-HA-S	70.8±0.36b	11.0±0.16c	15.6±0.23c	4.0±0.43bc	45.1±1.35c
B70-HA-S	69.5±2.41b	14.7±0.36b	21.2±0.52b	3.6±0.21c	52.3±3.75b
HA-S	69.1±2.38b	20.1±0.41a	29.1±0.59a	2.5±0.34d	57.8±2.12a
S	78.6±1.14a	0.5±0.19e	0.6±0.24e	5.4±0.25a	34.7±0.8e

B30-HA-S, blend 30% high amylose semolina and 70% semolina; B50-HA-S, blend 50% high amylose semolina and 50% semolina; B70-HA-S, blend 70% high amylose semolina and 30% semolina; HA-S, high amylose semolina; S, durum wheat semolina. Different letters in the same column indicate statistically significant difference ($p<0.05$). TS, total starch; RS, resistant starch; DS, damaged starch.

5.3.2 Characteristics of uncooked pasta samples

Table 5.4 shows the color and composition of uncooked pasta samples. Color evaluation evidenced a progressive decrease of b* values in high amylose pasta (24.4 in P-B30-HA-S and 21.4 in P-HA-S) that may be due to the higher proportion of bran fraction and duller color of high amylose flour compared to a durum wheat semolina, as also reported by De Arcangelis et al. (2021) and Sissons et al. (2020). Protein content was lower in high amylose pasta samples, and the same trend was registered with the total starch (TS) content which reached 69% d.w. on average in high amylose pasta, compared to 75.9% d.w. of the control sample. The inclusion of high amylose semolina in the formulation caused a significant increase in resistant starch (RS) fraction ($p<0.05$), reaching approximately 3% d.w. in P-B30-HA-S and 10% d.w. in P-HA-S, while negligible levels were recorded in control pasta. Nevertheless, compared to corresponding blends (Tab. 5.3) the resistant starch content in uncooked pasta samples was substantially halved, considering the values both as % d.w. and %TS. A higher proportion of starch damage, on average 3 times higher, was recorded in pasta samples compared with the respective blend/semolina.

Table 5.4. Color evaluation and composition of pasta samples.

PASTA	b*	PROTEIN (N×5.70)	TS	TDF	RS		DS	AMYLOSE
		%dw	%dw	% dw	%dw	% TS	% TS	%
P-B30-HA-S	24.4±0.87 b	12.9±0.00 b	72.0±0.05 b	4.3±0.32c	3.4±0.63 c	4.9±0.91b	13.2±0.32b	42.2±0.49d
P-B50-HA-S	23.6±1.22 bc	12.1±0.02 c	70.2±0.56 c	6.0±0.88b	5.4±0.52 bc	7.7±0.64c	11.7±0.69c	47.9±0.42c
P-B70-HA-S	22.9±1.10 c	11.3±0.01 d	70.5±1.11 c	7.3±0.65b	7.2±0.80 b	10.3±1.13b	9.4±0.88d	51.1±2.90b
P-HA-S	21.4±0.61 d	10.3±0.01 e	64.1±0.21 d	9.3±0.30a	9.8±0.05 a	15.3±0.16a	7.8±0.52e	58.0±0.78a
P-S	27.0±0.92 a	13.9±0.01 a	75.9±4.12 a	3.5±0.24d	0.5±0.01 d	0.7±0.01e	15.4±0.29a	37.5±2.19e

P-B30-HA-S, pasta 30% high amylose semolina and 70% semolina; P-B50-HA-S, pasta 50% high amylose semolina and 50% semolina; P-B70-HA-S, pasta 70% high amylose semolina and 30% semolina; P-HA-S, pasta 100% high amylose semolina; P-S, pasta 100% commercial semolina. Different letters in the same column indicate statistically significant difference ($p<0.05$). TS, total starch; TDF, total dietary fiber. RS, resistant starch; DS, damaged starch.

This can be explained by grinding of pasta samples for analysis, that increased this fraction and reduced particle size, thus affecting the penetration of amylase and resistant starch measurement (Li et al., 2014). Despite this, according to the resulted values, it can be stated that a consistent fraction of digestible starch was replaced by resistant starch in high amylose pasta, meeting in sample P-HA-S (15.3% TS) the requirements established by the EU Reg. 432/2012 on the health *claim* on resistant starch (14% total starch). Total dietary fiber considerably increased in proportion to the inclusion of high amylose semolina in the formulation, reaching the requirements to bear a nutritional *claim* on dietary fiber (3-6 g/100 g) according to Reg. EC 1924/2006 in all pasta sample.

5.3.3 Physico-chemical characteristics of cooked pasta and in vitro digestibility

Resistant starch content of cooked-to-optimum pasta samples (Tab. 5.5) spanned from 1.9% in P-B30-HA-S to 4.3% in P-HA-S compared to almost null content in P-S. More in detail, in our study, cooked pasta had a higher level of resistant starch (% d.w.) (Tab. 5.5) compared to uncooked samples, which could be due to retrogradaded amylose chains occurring during cooling of gelatinized starch (Haralampu, 2000). This allowed for a higher proportion of resistant starch over total starch. Equally, in Bresciani et al. (2021) cooking did not affect the nutritional quality and starch properties of high amylose pasta produced with pre-gelatinized corn flour. Similar results were obtained by Aravind et al. (2013) also with the incorporation of maize RS, while Gelencsér et al. (2008) found a marked reduction of resistant starch content attributable to the heat sensitivity of RS-rich material included in the formulation. Apparently, cooking did not influence starch digestibility of high amylose pasta, however the results of the

cooked samples were not in agreement with values of RS found in semolina. Potential discrepancies in this case may be due to gelatinization itself that increased enzymatic susceptibility of starch, thus reducing the resistant starch similarly to what happened in uncooked samples due to damaged starch. Nevertheless, samples presented 8.4-19.9% RS over total starch, coherently with the opportunity in cooked pasta (P-B70-HA-S and P-HA-S) to meet the EU Reg. 432/2012 requirements for the health *claim* on resistant starch "*Replacing digestible starches with resistant starch in a meal contributes to a reduction in the blood glucose rise after that meal*".

The assessment of starchy food digestibility is important in order to evaluate the amount of glucose release in the blood after the consumption of given food, and thus have an indication for the prevention of disease related to insulin resistance (Simonato et al., 2015). A good correlation was demonstrated (Goñi et al., 1997) between the data obtained with *in vivo* and *in vitro* methods, so the latter was used in this study to evaluate the extent of starch digestion. A similar relationship between *in vitro* and *in vivo* digestion values with rheological characteristics of cereal doughs was observed recently by Renoldi et al. (2021).

Figure 5.2 shows the kinetics of *in vitro* starch digestibility (0-180 min) for cooked-to-optimum pasta samples. The initial small amount of hydrolyzed total starch for all pasta samples is due to the presence of residual sugar in the cooked pasta prior to the action of enzymes. The incremental substitution of durum wheat semolina with high amylose bread wheat semolina led to a decrease of starch digestion rate. In particular, up to 30 min, the digestion is rapid and with little variability between high amylose pasta samples. After this time the curves of P-B30-HA-S and P-B50-HA-S overlap after 150 minutes of digestion, instead up to 60 minutes of digestion, the curves of P-

B70-HA-S and P-HA-S are similar, then pasta made with only HA-S shows a slower rate and the lowest percentage of hydrolyzed total starch.

These results could be explained by the presence of resistant starch that remains undigested and thus limits the starch hydrolysis (Tab. 5.5).

The glycemic index is considered a useful nutritional concept to classify foods in function of their ability to increase post-prandial insulin respond (Lucas-González et al., 2020). In table 5.5 the hydrolysis index (HI) and expected glycemic index (eGI) of cooked-to-optimum pasta samples are reported. Both values decreased as the percentage of high amylose semolina in pasta formulation increased. Although the degradation of starch granules, which occurs during pasta cooking, increases the susceptibility to amylolytic enzymes (Wang et al., 2015), in these samples, the presence of resistant starch reduces starch digestibility as indicated by lower eGI for high amylose pasta samples compared to control pasta.

Sissons et al. (2020) evaluated the effects on *in vitro* starch digestibility and *in vivo* glycemic response of spaghetti made with high amylose durum wheat semolina dried up to a maximum of 65°C, and they found a strong reduction of glycemic index in the genotype SBEIIa (amylose=58%) compared to control (38 vs 48), but not in the genotype SSIIa (amylose=44%). Other authors (Ang et al., 2020; Belobrajdic et al., 2019; Vetrani et al., 2018) have recorded a reduction of *in vivo* post-prandial glycemic response after the consumption of bread, noodles and rusks made with high amylose soft wheat flour. Improved glycemic response was also observed in pasta obtained from high amylose rice (Taddei et al., 2021).

Consumption of whole grain products promotes a lower risk of cardiovascular disease, type II diabetes, and cancer (Aune et al., 2016). Dietary fiber contributes to the reduction of starch digestibility in wholemeal pasta (Padalino et al., 2017), but

different authors (Gallo et al., 2020; Vignola et al., 2018) found a higher digestion rate for wholemeal pasta compared to semolina one, due to the weaker gluten network which enhances starch amylolysis. The study of Scazzina and coworkers (Scazzina et al., 2016) evidenced a variability of *in vivo* GI registered in semolina/wholemeal pasta, highlighting the possible role of processing and preparation in the recorded measurements.

In addition, Li et al. (2021) highlighted the contribution of protein-starch interactions to starch digestibility in high amylose flours characterized by a higher protein content compared to control wheat. In our study, despite the protein content of high amylose pasta samples was significantly lower than control pasta (13.9% d.w. in P-S compared to 11.7% d.w. on average for high amylose pasta samples), the starch granules amylolysis is markedly reduced in experimental samples, indicating that the slower starch digestion rate is exclusively due to the higher proportion of resistant starch. Furthermore, the limited granule disruption of high amylose starches during cooking contributes to the metabolic effect, by preserving the molecular structure of the whole matrix that acts as a physical barrier to the diffusion of α-amylases (Li et al., 2021).

Pasta is considered a low glycemic index food, but this study demonstrates that high-amylose wheat could be used to determine a further reduction of glycemic index, and thus improve the nutritional properties of pasta, eventually even compared to wholegrain products, due to the lower digestibility of starch. In addition, further research might also highlight the combined effect of amylose and a strong gluten network on the glycemic response of high amylose pasta (Ang et al., 2020).

Table 5.5. Total starch (TS) and resistant starch (RS) content, *in vitro* hydrolysis index (HI) and expected glycemic index (eGI) of cooked-to-optimum pasta samples.

SAMPLES	TS		RS			HI	eGI
	%	% d.w.	%	%d.w.	%TS		
P-B30-HA-S	22.0±1.61b	59.3±4.33b	1.9±0.13d	5.0±0.13d	8.4±0.58d	21.4±0.17b	51.4±0.09b
P-B50-HA-S	20.4±2.16b	54.6±5.80b	2.7±0.07c	7.2±0.19c	13.3±0.35c	20.4±0.13c	50.9±0.07c
P-B70-HA-S	21.5±0.76b	58.9±2.09b	3.4±0.30b	9.4±0.83b	16.0±1.41b	17.8±0.03d	49.5±0.01d
P-HA-S	22.0±0.53b	61.9±1.50b	4.3±0.02a	12.0±0.06a	19.4±0.10a	15.1±0.46e	48.0±0.25e
P-S	24.6±1.90a	69.2±5.34a	0.5±0.01e	1.5±0.02e	2.2±0.03e	25.6±0.11a	53.8±0.06a

P-B30-HA-S: pasta 30% high amylose semolina and 70% semolina, P-B50-HA-S: pasta 50% high amylose semolina and 50% semolina, P-B70-HA-S: pasta 70% high amylose semolina and 30% semolina, P-HA-S: pasta 100% high amylose semolina, P-S: pasta 100% semolina. Different letters in a column indicate statistically significant difference ($p<0.05$).

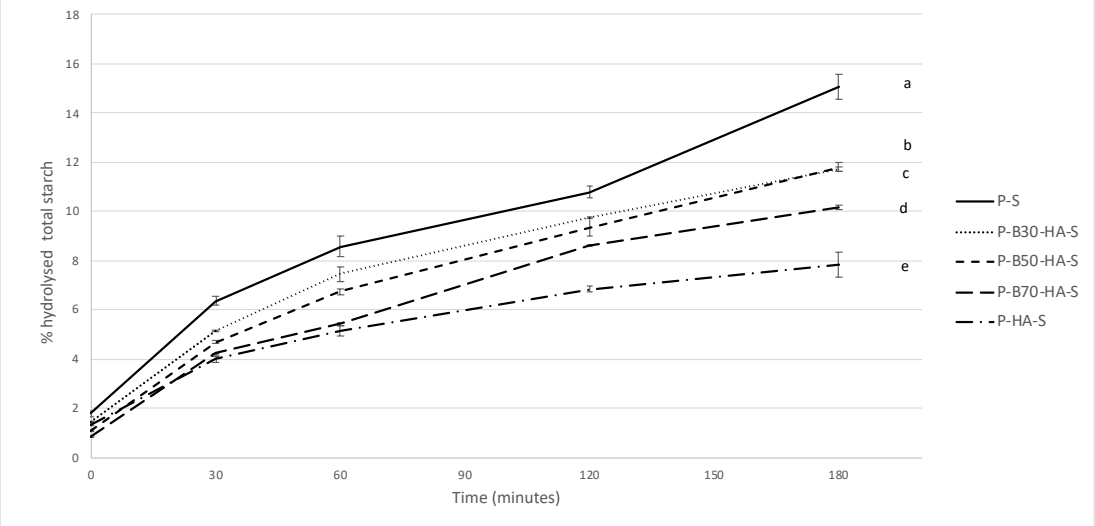

Figure 5.2: Total starch hydrolysis rate of cooked pasta samples. P-B30-HA-S: pasta 30% high amylose semolina and 70% semolina, P-B50-HA-S: pasta 50% high amylose semolina and 50% semolina, P-B70-HA-S: pasta 70% high amylose semolina and 30% semolina, P-HA-S: pasta 100% high amylose semolina, P-S: pasta 100% semolina. Different lower-case letters indicate statistically significant difference ($p<0.05$).

5.3.4 Cooking properties

The cooking losses of pasta (CL) (Tab. 5.6) increased markedly in P-B70-HA-S compared to control pasta reaching 10 g/100 g in P-HA-S, which are on average similar to values reported by Sissons et al. (2020) using a high amylose durum wheat semolina. This result confirm a typical behavior of high amylose pasta, due to the higher ability of amylose to leach in cooking water (Hazard et al., 2015; Hogg et al., 2015; Soh et al., 2006). This is further corroborated by Sharma et al. (2002), who found that instead reported a lower cooking loss in samples obtained from waxy (low-amylose) wheats. Despite gluten quality was comparable between high amylose semolina and commercial semolina (Tab. 5.2), in our study, the negative impact of high amylose semolina on CL might be supported also by its lower protein and gluten content than control semolina, causing a weaker gluten matrix entrapping starch granules during cooking.

Water absorption index (WAI) is closely related to the structure of starch, fiber and gluten network. Variation of WAI is recorded between P-B30-HA-S and P-B50-HA-S pasta samples with values that are significantly different to control pasta (129%) (Tab. 5.6). A higher amylose proportion further decreased water absorption, reaching 119% and 110% in P-B70-HA-S and P-HA-S samples, translating into significant differences in the swelling behaviors of starches. These results highlight that amylose may prevent water absorption during starch gelatinization, as already described in flours (Botticella et al., 2018; Jaksics et al., 2020) and in pasta samples by other researchers (Hazard et al., 2015; Hogg et al., 2015; Li et al., 2021).

Textural attributes such as firmness, liveliness and starch release are discriminating for consumer preferences. The inclusion of high amylose flours in pasta formulations

substantially affected starch release and liveliness (Tab. 5.6), leading to a poor quality of pasta made with 100% HA-S (total score= 32). These factors mainly describe the surface properties of pasta samples, while lower impact were perceived with the firmness attribute that instead relates to the intrinsic structural properties of pasta (Cubadda et al., 2007). The increase of surface stickiness of experimental pasta could be due to the higher migration of amylose on the surface (Cubadda et al., 2007; Sissons, 2008). A lower high amylose flour proportion, mainly in P-B30-HA-S and P-B50-HA-S, markedly improved the quality of high amylose pasta, that, however, resulted still lower than control pasta (total score= 68).

For a better textural investigation, texture analysis was adapted for cutting tests on cooked-to-optimum pasta samples. The increased amylose proportion in blends caused a decrease of both the maximum force required for samples cut (hardness), and cutting work (firmness) (Fig. 5.3). Particularly, hardness spanned from 519 g to 376 g in high amylose samples, while reached 583 g in control pasta. A similar trend was observed for firmness parameter, as P-HA-S reached the lowest value of 280 g s, while in control pasta it resulted 505 g s. These results are not in agreement with previous studies on high amylose spaghetti (Hazard et al., 2015; Soh et al., 2006) and noodles (Heo et al., 2012; Li et al., 2021; Newberry et al., 2018) cooked at both OCT and for a fixed time. In fact, Hazard et al. (2015) cooked high amylose spaghetti samples for a fixed time (12 min) resulting in higher pasta firmness compared to wildtype line. Soh et al. (2006), on the other hand, measured firmness in pasta cooked at OCT registering a trend of positive correlation with amylose content, significant only at 55% amylose level. Li et al. (2021) produced noodles cooked for a fixed time of 10 min. Also in this case the limited swelling ability of high amylose starch resulted in higher firmness. Concurrently, the study Heo et al. (2012) reported a negative relationship between

hardness and waxy flour proportion in noodles prepared from reconstituted flours, in agreement with Vignaux et al. (2005). Our results are instead in line with the study of Sissons et al. (2020). Despite the lower swelling/water absorption ability of amylose should cause a higher pasta firmness, other authors did not find any positive trends in pasta samples enriched in RS (Aravind et al., 2013; Sozer et al., 2007). It should be noted, however, that unlike Sissons et al. (2020), a bread wheat semolina is used with a lower protein content than control flour. We therefore postulate that lower protein quantity in high amylose blends, weaker gluten network and protein-starch interactions caused a lower pasta firmness (Sissons et al., 2005). Overall, our data proved a negative correlation (Tab. 5.7) between RS and textural properties of cooked pasta (r=-0.99).Given the obtained evidences on the role of amylose content on pasta cooking quality, we expect that processing improvement with adequate formulations (e.g. with the addition of vital gluten) and pasta drying conditions (HT- High Temperature drying) (Cubadda et al., 2007; D'Amico et al., 2015; Samaan et al., 2006) might further enhance the cooking performance of high amylose bread wheat semolina pasta.

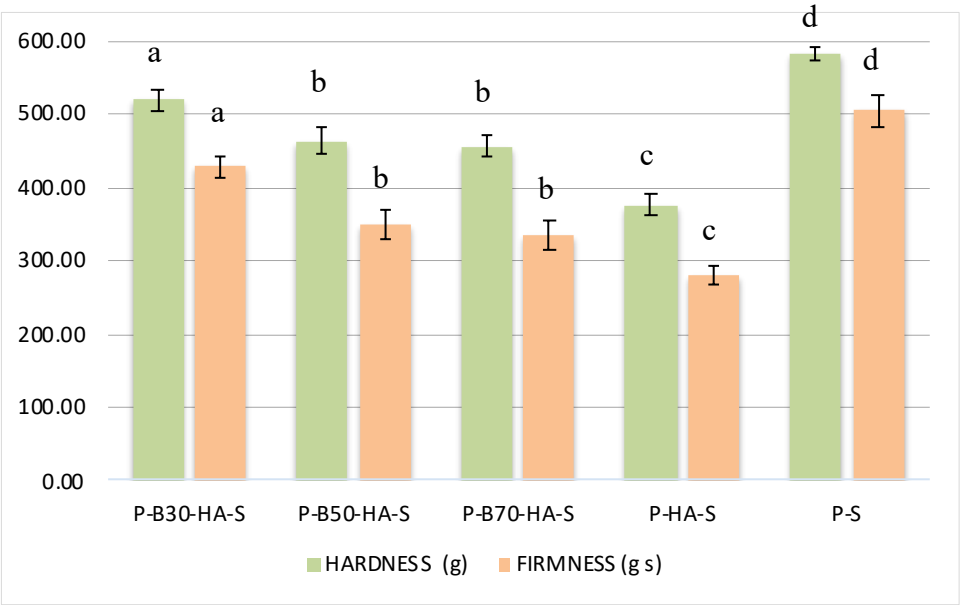

Figure 5.3. Textural properties of pasta samples. P-B30-HA-S: pasta 30% high amylose semolina and 70% semolina, P-B50-HA-S: pasta 50% high amylose semolina and 50% semolina, P-B70-HA-S: pasta 70% high amylose semolina and 30% semolina, P-HA-S: pasta 100% high amylose semolina, P-S: pasta 100% semolina. Different letters indicate statistically significant difference ($p<0.05$).

Table 5.6. Optimum cooking time and cooking quality of pasta samples

SAMPLES	OPTIMUM COOKING TIME	COOKING QUALITY						
		CL	WAI	SI	Firmness	Starch release	Liveliness	Total score
	min	%	%	g water/g dry pasta				
P-B30-HA-S	07:30	6.2 ± 0.64 d	123±1.3 c	1.66±0.035 d	55	61	60	59
P-B50-HA-S	07:30	6.3±0.11 cd	127± 0.2 b	1.74±0.000 a	55	60	59	58
P-B70-HA-S	07:30	8.0±0.55 b	119±0.01 d	1.70±0.028 c	55	54	55	55
P-HA-S	07:10	10.1±0.46 a	110±2.8 e	1.67±0.035 d	55	20	20	32
P-S	07:50	6.5±0.05 c	129±0.4 a	1.73±0.028 b	65	70	70	68

CL: cooking loss, WAI: water absorption index, SI: swelling index. P-B30-HA-S: pasta 30% high amylose semolina and 70% semolina, P-B50-HA-S: pasta 50% high amylose semolina and 50% semolina, P-B70-HA-S: pasta 70% high amylose semolina and 30% semolina, P-HA-S: pasta 100% high amylose semolina, P-S: pasta 100% semolina Different letters in the same column indicate statistically significant difference ($p<0.05$).

Table 5.7. Linear correlation coefficient (r) for cooking and textural properties, and resistant starch content of cooked-to-optimum pasta samples.

	CL	WAI	SI	OCT	RS	FIRMNESS
CL	1	-0.93*	-0.44	-0.76	0.80	-0.74
WAI		1	0.70	0.89*	-0.85▪	0.78
SI			1	0.60	-0.40	0.29
OCT				1	-0.93*	0.91*
RS					1	-0.99**
FIRMNESS						1

Significant at ** $p < 0.01$; * $p < 0.05$; ▪ $p< 0.1$. CL: cooking loss, WAI: water absorption index, SI: swelling index, OCT: optimum cooking time, RS: resistant starch.

5.4 Conclusions

In this study a semolina-type flour from high amylose bread wheat was used to formulate pasta, providing a substantial enhancement of the nutritional quality of the products compared to a durum wheat pasta. In fact, the resistant starch fraction ranged from 4.7 to 15.3% of total starch, compared to the almost null content in durum wheat pasta, allowing the formulation obtained with 100% high amylose semolina to bear the health claim on resistant starch (Reg. EU 432/2012). The employment of high amylose semolina positively impacted on the starch hydrolysis rate compared to a pasta obtained with durum wheat semolina, with a considerable decrease of expected glycemic index which could promote glucose control in healthy individuals and also in adults with diabetes. The obtained pasta evidenced an acceptable cooking quality, nevertheless, a major improvement of textural and nutritional properties can be achieved by increasing the protein fraction with the addition of vital gluten, and by implementing processing conditions with high/medium temperature drying.

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General conclusions

This PhD thesis showed the possibility to use high amylose bread wheat for the production of innovative cereal-based products (semolina-like flour and pasta).

The first relevant result of this work was the obtaining of high amylose flours from milling of high amylose bread wheat, with deep chemical-physical differences compared to normal starch wheat.

The developed innovative flours showed different nutritional and rheological characteristics that could be employed for the production of healthy cereal products, thanks to the higher content of resistant starch and fibre.

In this work the produced semolina-like flour was used for the preparation of pasta that is a staple food consumed all over the word and thus could be a valid vehicle for delivering human health benefits.

In order to meet both nutritional as well as healthy and technological characteristics, appropriate blends were studied between high amylose semolina and durum wheat semolina. The result was the production of pasta samples with higher content of resistant starch, satisfying the requirements of the health *claim* on resistant starch enlisted in EU Reg. 432/2012: "*replacing digestible starches with resistant starch in a meal contributes to a reduction in the blood glucose rise after that meal*", with a substitution of at least 14% of total starch with resistant starch.

After the evaluation of *in vitro* kinetic starch digestion of cooked pasta samples, a decrease of starch digestion rate was recorded in all high amylose pasta samples, confirming the role of resistant starch in limiting the starch hydrolysis.

Moreover, foods rich in resistant starch contribute to the fibre intake and thus can help fill the fibre quantity and quality gap reaching also the nutritional *claim* on fibre enlisted in EC Reg. 1924/2006.

However, the success of this strategy hinges on several prerequisites. For example, consumer acceptance of pasta enriched in resistant starch is obviously paramount and in this study it was assessed through the evaluation of cooking quality: for pasta samples an improvement of textural and cooking properties is advisable with appropriate formulations (e.g. addition of vital gluten) and processing (e.g. high temperature drying), since any change in wheat grain composition must not negatively affect eating quality of foods formulated to contain this ingredient.

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