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MACROMOLECULAR CHARACTERISTICS AND BREAD MAKING PROPERTIES OF ITALIAN WAXY WHEAT LINES

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PhD Thesis of:
Rosita CARAMANICO

Tutor:
Prof. M. Ambrogina PAGANI

Coordinatore:
Prof. M. Grazia FORTINA

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SUMMARY

This PhD thesis aims to analyse the waxy (amylose-free) wheat (*Triticum aestivum* L.) properties and the role in the textural characteristics and staling kinetics of bread and bakery products. Moreover, *waxy* genes effects on qualitative and agronomic characteristics of autochthonous lines selected by a breeding project aimed to obtaining *waxy* genotypes suitable for cultivation in Italy have been evaluated. The present PhD thesis, therefore, was divided into six chapters:

1. STATE OF THE ART: WAXY WHEAT ORIGINS, CHARACTERISTICS AND APPLICATIONS

Food industry is increasingly interested in waxy (amylose-free) starch because its application to bakery products seems to be able to naturally induce retardation of staling and to extend shelf-life. The starch retrogradation is indeed believed to be the major cause of bread staling and amylose content is assumed to be the main contributor to bread firming. This section offers an overview related to waxy wheat properties and briefly describes how first waxy wheat varieties have been obtained. Moreover, the main composition and thermal properties of waxy starch are illustrated together with the main waxy wheat applications in food products.

2. AGRONOMICAL AND TECHNOLOGICAL PROPERTIES OF ITALIAN WAXY WHEAT

The high influence of different environments on wheat productivity and quality may in principle impede that waxy wheat lines produced in other countries can be successfully cultivated in Italy. In the perspective of developing waxy wheat lines suitable for cultivation in Italy, various research activities have been accomplished since ten years in this country. Aim of the present study has been the evaluation of agronomic and technological performances of a set of 18 Italian waxy wheat lines (IWWL), derived from a breeding program started in 2000 and set up at CRA-SCV (Italy) from partial-waxy cultivars belonging to Italian germplasm.

This study shows that, despite the supposed high influence of different environments on wheat productivity and quality, agronomical tests repeated during three years did not allow detecting significant differences between IWWL and two American waxy wheats as well as between IWWL and not waxy wheat in relation to their agronomical characteristics. On the contrary, important differences related to technical characteristics have been observed. Almost all IWWL showed higher bread making qualities with respect to the Amirican waxy and non-waxy wheats used as controls.

Six out of eighteen IWWLs analysed showed particularly good performances. In particular, these lines resulted characterized by higher values of gluten index, SDS sedimentation volume, farinograph stability and bread specific volume. Some of these six lines will be further tested during the next growing seasons in three different locations in order to confirm the results achieved.

3. PHYSICOCHEMICAL AND STRUCTURAL CHARACTERISTICS OF STARCH FROM ITALIAN WAXY WHEATS

Starch is the most abundant constituent in wheat flours and plays an extremely important role in their pasting properties. The differences in starches amylose/amylopectin ratio result in different pasting properties of flour, and, therefore, in the texture and quality of bakery products.
This study aimed to investigate some of the main physicochemical and structural characteristics of waxy starch. In particular, the analyses have focused on thermal and pasting properties of flour from some waxy wheat lines adapted to the Italian environmental conditions, which have been compared with two American waxy wheats and one non-waxy wheat, taken as reference.
Results allowed to highlight how the low amylose content of all IWWL (resulted markedly below 1.7% d.b) influences the characteristics of both suspension and dough systems. Waxy wheat samples resulted having a higher number of small starch granules, often with deformities and small cracks with respect to non-waxy wheats. Regarding starch gelatinization, this phenomenon was observed at higher temperatures (as estimated by DSC analyses) in waxy starch with respect to non-waxy starch. Nevertheless, gelatinization temperature is strongly affected by the conditions applied (e.g. water availability, presence/absence of shear-stress, etc.) in the approach used for its evaluation. As the retrogradation extent resulted highly reduced in all the IWWL flours, these types of flours could be usefully exploited for baking processes.

4. PROTEIN CHARACTERIZATION AND RHEOLOGICAL PARAMETERS OF ITALIAN WAXY WHEAT LINES

Except for starch properties, there is still limited information about the quality characteristics of complete waxy wheat cultivars and there is a need to better understand how waxy wheat protein composition could affect the physical properties of dough and bakery products. This study analysed the molecular characteristics of proteins from IWWL, comparing the results with those obtained for two samples of American waxy flour and two non-waxy flours with good bread-making quality.
The protein content of IWWL flours resulted on average quite high, ranging from 12.0% to 15.4%. The composition observed for the high-molecular weight-glutenin subunits (HMW-GS) of IWWL was generally associated with medium bread-making quality and the electrophoretic analysis indicated that all IWWLs considered have a similar gliadin composition. Differential-solubility data did not show any significant difference between waxy and non-waxy proteins and also the accessibility of sulfhydryls groups seemed to be comparable between IWWL and non-waxy wheat proteins. Front-face fluorescence analyses allowed instead to highlight an interesting difference between IWWL and non-waxy wheat proteins: in fact, dough from IWWL flours needed more water to completely hydrate their proteins, probably as a consequence of the relevant water-retaining capacity of waxy wheat starch. Moreover, the peculiar properties of waxy wheat starch seemed to affect the interactions between protein hydrophobic regions and amylopectin partially solvated regions. Finally, non-waxy flour dough resulted having a much more solid elastic-like behavior with respect to waxy flour dough whereas IWWL flour dough resulted having higher elasticity and consistency indices with respect to American waxy dough.

5. BREAD MAKING CHARACTERISTICS AND STALING KINETICS OF ITALIAN WAXY WHEAT LINES

Waxy wheat is considered to have superior functional properties for bread-making, resulting in the formation of a soft bread-crumb and improving bread shelf-life due to staling retarding. This study focused on the Italian context and considered three Italian waxy wheat lines (IWWL). The properties of dough and the quality of fresh bread obtained by these lines were compared with those of two American waxy wheat samples and a non-waxy flour with high bread-making quality. Bread-making quality has been analysed also in relation to shelf-life.

The specific volume of bread samples from IWWLs resulted significantly higher than that of the controls: in fact, the estimated mean area for the large cells of crumb resulted significantly higher for most of the IWWL samples with respect to non-waxy samples. As expected, breadcrumb firmness of all samples from IWWLs resulted lower than commercial flour breadcrumb firmness for 7 days after baking at least. Among IWWLs, bread from wx123 line achieved the highest volume. Its breadcrumb presented the lowest firmness while its breadcrumb moisture was about 3% over that of the commercial flour bread after 7 day storage. During this storage time, the moisture decrease in wx123 bread resulted of 27% against 35% registered for the samples from commercial flour, whereas the decrease in water activity (6%) was the same among all bread samples. This different behavior could partially explain the lower bread firmness detected for wx123 samples. In general, the waxy wheat lines adapted to the Italian environmental conditions showed better bread-making qualities with respect to American standard waxy lines.

6. INFLUENCE OF FLOUR BLENDS ADDED WITH ITALIAN WAXY WHEAT LINE ON BREAD MAKING QUALITY AND STALING KINETICS

Bread samples from waxy flour were typically characterized by higher volume and retarded staling; nevertheless dough from waxy flour had higher stickiness (and consequently a lower machinability) with respect to non-waxy flour. This study aimed at investigating how this negative characteristic could be decreased by using blends of "normal" wheat flour and IWWL flour, without compromising the other above mentioned good bread-making qualities. Flour blends contained 20% and 40% of commercial flour from non-waxy wheat; the dough and bread properties were compared to American waxy wheat lines used as reference. This approach allowed reducing adhesiveness increasing farinograph stability and dough consistency. The starch retrogradation kinetics wasn't affected by the presence of commercial flour, as assessed by measuring the firmness of gels stored at low temperatures for long time periods.

As regards dough rheological properties, the values registered for the elastic modulus and the viscous modulus in case of waxy dough samples with blends of non-waxy flour varied within the same ranges observed in case of dough samples from pure waxy or pure non waxy flours confirming a solid elastic-like behavior with the elastic modulus prevailing over the viscous modulus also for flour blends dough samples.

Concerning bread properties from IWWL flour blends, the specific volumes resulted significantly higher than those of bread samples from non-waxy and American waxy flours. Crust color did not significantly change with respect to breads from 100% IWWL flour excepting for crust redness. The lightness of crumbs increased significantly but remained significantly lower than that of breadcrumb from pure non-waxy flour.

As regards bread cells area, the addition of commercial flour in IWWL flour did not determine on average a significant increase in the ratio between cell area and total area of bread cells due to an increase in the density of small cells.

Breadcrumbs from blends with 20% of non-waxy flour resulted having good bread-making qualities and maintained low firmness values till 7 days of storage.

PREFACE

Waxy wheats are characterized by a very low amylose content (Hung et al., 2007) due to the absence of the three isoforms of the granule-bound starch synthase (GBSS-I) responsible for the biosynthesis of starch amylose (Preiss and Sivak, 1996). So far no wheat lines that are “naturally” lacking of all the three GBSS isoforms have been identified worldwide and all existing completely waxy wheats are generated by mating the offsprings from the cross between partial-waxy cultivars. Considering that starch retrogradation is believed to be the major cause of breadcrumb firming, commonly referred to as bread staling, and that the amylose is assumed to be the main contributor to bread firming (Hung et al., 2007), food industry is lately increasingly interested towards waxy (amylose-free) starch because in principle it should allow avoiding using common additives to bakery formulations to extend the shelf-life of baked products. Graybosh et al. (2003) analysed the influence of environmental conditions on waxy wheat productivity and quality and concluded that significant variations occurred for most of the quality traits of flours from grains produced in different environments. For this reason it is hence unlikely that waxy wheat lines produced in other countries can be successfully cultivated in Italy and waxy wheats obtained from traditional crossing starting from partial-waxy autochthonous landraces have to be employed.

The final objective of this PhD project was the identification and the evaluation of macromolecular characteristics and bread making properties of Italian waxy wheat lines obtained as above mentioned. The following main phases have been considered in order to reach this objective. The results obtained during each phase mainly represent the starting point of the following one.

Phase 1: The first part of the work consisted in an overview related to existing studies on waxy wheat characteristics, waxy starch properties together and main waxy wheats application in food products.

Phase 2: The aim of this part of the work consisted in the agronomic and technological characterization of a set of 18 completely waxy lines grown in field trials at the *Unità di Ricerca per la Selezione e la Valorizzazione delle Varietà Vegetali* (CRA-SCV) in S. Angelo Lodigiano (Italy) and previously obtained in greenhouse by a traditional breeding programme started in 2000 and set up at CRA-SCV. This allowed to select the best waxy lines that could be proposed for cultivation in Italy.

Phase 3: This part of the work aimed to investigate some of the main physicochemical and structural characteristics of the starch fraction of the Italian waxy wheat lines (IWWL) selected. These characteristics have been analysed by using two waxy wheat varieties (selected in the USA[1] but grown in Italy at CRA-SCV and called “American waxy wheats” in the following) and non-waxy wheat flours as terms of comparison in order to highlight possible peculiarities of waxy lines cultivated in Italy. Analyses accomplished during this phase have been performed in collaboration with Prof. Dimitrios Fessas (DISTAM; Università degli Studi di Milano), Prof. Franco Faoro (DIPROVE; Università degli Studi di Milano), Prof. Mara Lucisano (DISTAM; Università degli Studi di Milano).

[1] See Morris & Konzak (2001)

Phase 4: During this phase the molecular characteristics of proteins from IWWLs have been analysed and it has been assessed how these characteristics affect the physico-chemical and rheological properties of related dough. The analyses accomplished have been performed in collaboration with Prof. Franco Bonomi and Prof. Stefania Iametti (DISMA; Università degli Studi di Milano)

Phase 5: This part of the work consisted in evaluating the properties of dough and the quality of bread (with particular attention to product shelf-life) obtained from the IWWLs and comparing the results with the bread-making performances of the American waxy wheat varieties and of a high bread-making quality non-waxy flour.

Phase 6: The final part of the work aimed to investigating how the poor handling properties and the stickiness observed for the dough from the IWWLs selected could be decreased by using flour blends with commercial non-waxy flour without compromising the positive results associated with bread storage. This part of the word was carried out in collaboration with Prof. Mara Lucisano (DISTAM; Università degli Studi di Milano)

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1. STATE OF THE ART: WAXY WHEAT ORIGINS, CHARACTERISTICS AND APPLICATIONS

1.1 INTRODUCTION

Starch is the principal component of wheat grain representing about 75% of grain weight (Stone & Morell , 2009). It contributes in important ways to determine appearance, structure and quality of food products. The two glucose polymers that constitute starch (amylose and amylopectin) are chemically similar, but differ in the degree of branching of D-glucosyl units, which are the core of these molecules. Amylose is an essentially linear molecule, consisting of α(1-4)-linked D-glucosyl units with a degree of polymerization (DP) in the range 500-6000 glucose residues. Amylopectin also contains α(1-4) d-glucosyl chains, but branches occur every 20±25 residues due to the presence of α(1-6) linkages. Amylopectina is a very large, highly branched chain molecule with a DP ranging from $3x10^5$ to $3x10^6$ glucose units.
The amylose/amylopectin ratio differs among starches, but typical amylose and amilopectin levels in bread wheat are 25-28% and 72-75% respectively (Van Hung et al., 2006).

In plants, starch is synthesized in specialized organelles known as amyloplasts. Within the amyloplasts, amylose synthesis seems to be solely accomplished by the granule-bound starch synthase (GBSS, EC 2.4.1.21), also known as the `waxy' protein (Graybosh, 1998). The granule-bound starch synthase (GBSS-I or Waxy protein) is responsible for the biosynthesis of starch amylose. As demonstrated by Sivak & Preiss (1995), both biochemical and genetic evidence indicate that GBSS is the major enzyme responsible for amylose synthesis.

The term `waxy' was first used for amylose-free mutants of maize, and refers to the waxy appearance of the endosperm of dried kernels, as opposed to the flinty or translucent appearance of normal (wild-type) kernels (Boyer & Hannah, 1994).

1.2 WAXY WHEAT ORIGINS: WAXY GENES AND WAXY PROTEINS

The identification of spontaneously occurring waxy mutants is precluded by the genetic structure of wheat. Wheat is a member of the grass tribe Triticeae and the base chromosome number of this group is x=7.
Bread wheat (*Triticum aestivum* L.), an allohexaploid, 2n=6x=42, contains three nearly identical sets (A, B and D genomes) of chromosomes inherited from three ancestral diploid species. Each genome consists of seven pairs of homologous chromosomes. Each chromosome pair is genetically similar to one specific chromosome pair of each of the two remaining genomes. Hence, wheat chromosomes may be further divided into seven "homoeologous" groups; the location of genes on and the structure of each member of these homoeologous groups is virtually identical (Graybosh, 1998). The genetic *loci* (wx) containing the genes encoding bread wheat GBSS are found on chromosomes 7A (wx-A1), 4A (wx-B1) and 7D (wx-D1) (Yamamori et al. 1994). The 4A *locus* originally was located on chromosome 7B; however, during the evolution of wheat, a reciprocal translocation occurred, resulting in an exchange of

genetic materials between chromosomes 7B and 4A (Yamamori et al. 1994). Hence, this *locus* is still designated wx-B1 despite its existence on an A chromosome.
Spontaneously occurring waxy bread wheats could have arisen only through either the simultaneous mutation of all three wx *loci* to render them non functional, or the chance combination of independent mutations through random cross-pollination. The probability of either event occurring is exceedingly low. Nevertheless researchers have now succeeded in developing waxy (amylose-free) wheats through hybridization of wheats carrying null (non-functional) alleles. In addition, the triplicate nature of the bread wheat waxy gene system has allowed the identification of `partial waxy' wheats, or wheats with reduced amylose content (Graybosh, 1998).

In bread wheat there are three waxy proteins designated as Wx-A1, Wx-B1 and Wx-D1 with respective molecular weight 60.1, 59.2 and 59.0 kDa, and encoded by three genes at the wx-A1, wx-B1 and wx-D1 *loci*, located on 7AS, 4AL and 7DS chromosome arms, respectively (Preiss et al., 1996; Chao et al., 1989, Fujita et al., 1996, Nakamura et al., 1993).

Nakamura et al. (1992) developed a modified sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE) system that allowed simultaneous one-dimensional separation of the three GBSS isoforms.

However, the three GBSS can be present in different combinations in wheat cultivars. Yamamori et al. (1994) have found various cultivars with null alleles for each of the three controlling *loci*. Null alleles at the Wx-A1 were common in wheats from Japan, Korea and Turkey, while a high frequency of Wx-B1 null alleles was detected in Australia wheats and only one line from China, ‘BaiHuo’, was found to carry a null alleles at the Wx-D1 *locus*. There is a correlation between the presence of Wx null alleles and amylose content across cultivars (Miura & Tanii, 1994; Yamamori et al., 1992), and effects on modifying amylose content of the three Wx genes are different. The Wx-B1b allele reduces the largest amylose through the lack of the Wx-B1 protein in comparison with the other null alleles, Wx-A1b and Wx-D1b (Miura & Sugawara, 1996; Miura, Tanii, Nakamura, & Watanabe, 1994). In contrast, the wild-type Wx-B1a predominates for amylose synthesis capacity, followed by Wx-D1a and Wx-A1a (Miura, Araki, & Tarui, 1999). Only in case of three null alleles (waxy wheat) the amylose content falls below 3% (Van Hung et al., 2007).

The world’s first waxy wheat was completely produced in Japan by Nakamura et al. (1995) using traditional hybridizations between the Wx-D1 single null line BaiHuo and the Wx-A1/Wx-B1 double null line ‘Kanto 107’ resulting in progeny that lacked of all isoforms of GBSS and had no starch amylose. Later, two additional methods for producing waxy wheat have been developed. Yasui et al. (1997) recovered two waxy wheat lines after treating seed of the double null line Kanto 107 with the mutagen ethyl methane sulphonate, while Kiribuchi-Otobe et al. (1997) identified five waxy wheat lines from a doubled haploid breeding program designed to move rapidly a low amylose characteristics from a mutant line to adapted genetic backgrounds.

Considering the high influence of the environment on productivity and product quality (Graybosh, 2003) waxy wheat lines produced in Japan and in other countries cannot be cultivated in Italy because of the different environmental conditions.

1.3 CHARACTERISTICS OF WAXY WHEATS

Starch granule is composed of amylose and amylopectin in a semi-crystalline form (Donald et al., 1997). The starch granule can be defined in terms of alternating amorphous and semi-crystalline growth rings or shells with a radial thickness of 120–400 nm (Buleon et al., 1998; French, 1984). The amorphous shells are less dense and contain amylose and probably less ordered (not crystalline) amylopectin, while the semi-crystalline shells are composed of alternating amorphous and crystalline lamellae of about 9–10 nm. The latter is made up of amylopectin double helices packed in a parallel fashion, while the former consists of the amylopectin branching regions (and possibly some amylose) (Jenkins et al., 1993).

Wheat starch granules exist as a bimodal population of large`A-type' and smaller `B-type' granules (Evers & Bechtel, 1998)

Waxy wheats exhibit similar amylopectin structure to that of non-waxy wheat starch and no substantial differences in the chain length distribution and degree of polymerization of amylopectin chains can be detected, indicating that waxy character has little influence on the chain-length distribution profiles of amylopectin molecules (Hayakawa et al., 1997; Yasui et al., 1996). X-ray diffraction patterns show that waxy starch exhibits A-type crystal and has higher crystallinity than non-waxy wheat starch due to higher percentage of amylopectin (Fujita et al., 1998; Hayakawa et al., 1997).

Thermal properties of the waxy wheats are determined by using differential scanning calorimetric (DSC) and waxy wheat starch shows only one peak for starch gelatinization indicating no evidence of amylose-lipid complex in waxy starch. Gelatinization temperatures and enthalpy of waxy wheat starch are significantly higher than those of non-waxy wheat starch (Fujita et al., 1998, Hayakawa et al., 1997; Yasui et al., 1996). These evidences indicate that waxy wheat starch with predominant amylopectin requires higher energy for gelatinization caused by its higher crystallinity as compared to non-waxy and high-amylose wheat starches.
Waxy wheat starch also exhibits high resistance to retrogradation during storage. During 3 weeks of storage, enthalpy of waxy wheat starch gels change little, while those of non-waxy wheat starch gels increase markedly (Hayakawa et al., 1997).

Amylopectin structure of waxy wheats is similar to that of wild-type and partial waxy wheats. No differences in amylopectin branching frequencies (Hayakawa et al, 1997) or degree of polymerization of amylopectin chains (Hayakawa et al, 1997; Yasui et al., 1996) are observed. Waxy wheat starch has little integral starch lipid, no doubt due to the absence of amylose with a subsequent loss of the amylose-lipid inclusion complexes that contain the majority of starch lipids (Yasui et al., 1996). X-ray diffraction analysis of starch gels (Hayakawa et al, 1997) during retrogradation shows very little increase in crystalline areas of waxy wheat starch. DSC analysis (Hayakawa et al, 1997) also demonstrats a marked resistance of waxy starch to retrogradation. To date, studies of the functional properties of waxy wheat starch have been limited to Brabender Visco Analyser (BVA) and Rapid Visco Analyser (RVA) characterization of starch viscosity, and, some conflicting results have been reported. Kiribuchi-Otobe et al. (1997), using an RVA, found that waxy wheat starch reaches a peak viscosity at 84 °C, 10°C lower than normal wheat starch. In addition, waxy wheat starch had a higher peak viscosity, but lower setback, than normal wheat starch.

1.4 WAXY WHEAT APPLICATIONS

Food industry is lately increasingly interested towards waxy (amylose-free) starch because its application to bakery products seems to be able to naturally induce retardation of staling and to extend shelf-life (Van Hung et al., 2007). "Staling" is indeed a significant phenomenon restricting the shelf life and acceptability of bread and is defined as:

a term which indicates decreasing consumer acceptance of bakery products caused by changes in crumb other than those resulting from the action of spoilage organisms (Batchel et al 1953).

The starch retrogradation is believed to be the major cause of bread staling and its amylose content is assumed to be in particular the main contributor to bread firming.
At least three US patents (Alexander, 1996; Furcsik, 1992; Zallie et al., 1986) describing the use of waxy starch as a means of extending the shelf-life of baked goods have been granted. Staling of baked goods results indeed in a considerable loss of revenue for bakers and supermarket chains. So far shelf-life extenders such as lecithin (Stampfli & Nersten, 1995), α-amylases (morgan et al. 1997; Champenois et al. 1999), Maltodextrins (Gerrard et al. 1997; Defloor and delcour 1999), lipids (Collar et al. 1998), surfactants (Rao et al. 1992; Faheid and Ragab 1996, Stauffer, 2000), shortenings or emulsifiers (Armero and Collar 1998, Forssell et al. 1998, Genc et al. 2000) are among the most common additives to bakery formulations.

One important waxy wheat application is also that by which waxy wheat is used as a blending wheat to develop flours with specific amylose contents. These flours are used for example to produce *Udon noodles* and *tortillas* whose quality is related to both starch swelling properties and amylose content (Baik et al., 2003; Bhattacharya et al., 2002; Waniska et al., 2002). Waxy wheat flour is also used for chilled and frozen dough products because the slow rate of starch retrogradation can be used to offset the decline in product quality during storage.
This flour is also often employed to improve textural properties and volume of extruded products like snack foods and breakfast cereals (Wang, 1997).

In theory, waxy wheat starch could be used in the same types of food and industrial applications currently utilizing waxy maize starch. Waxy maize starch is a preferred substrate for the development of 'modified' starches for food, papermaking and adhesive industries (White, 1994), and is a superior substrate than normal maize starch for the production of maltodextrins (Alexander, 1992). Fat replacement also is a common application of waxy or modified waxy maize starch (Alexander, 1992). Waxy starches used in the food industry are typically chemically modified (White, 1994).

In 1998 Graybosh at el. observed that wheat starch is produced essentially as a by-product of the wheat gluten industry and that coupling the waxy starch character with high-protein strong gluten wheats could allow the production of waxy wheat starch at a price that was competitive with that of waxy maize. Apparently this did not yet happen.

Recent studies (Smith et al., 2006; Lacerenza et al., 2008) recommend the employment of waxy wheat as a feedstock for fuel ethanol production, because waxy cultivars show an overall higher conversion efficiency during fermentation with respect to nonwaxy cultivars (Zhao et al. 2009).

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2. AGRONOMICAL AND TECHNOLOGICAL PROPERTIES OF ITALIAN WAXY WHEAT

2.1 STATE OF THE ART

Starch is the main component of wheat grain. It plays an important role in appearance, structure and quality of cereal products. The two glucose polymers that comprise starch, amylose and amylopectin, are chemically similar, but differ in the degree of branching of D-glucosyl units, which are the core of these molecules. The amylose/amylopectin ratio differs among starches, but typical amylose and amylopectin levels in bread wheat are 25-28% and 72-75% respectively.

The granule-bound starch synthases (GBSSs) are the major enzymes responsible for amylose biosynthesis. In many species, including wheat, two isoforms have been identified, GBSSI and GBSSII. GBSSI, or waxy protein, appears to be mostly confined to storage tissues, while GBSSII, encoded by a separate gene, is thought to be responsible for amylose synthesis in leaves and other non storage tissues which accumulate transient starch (Vrinten & Nakamura, 2000). In bread wheat there are three waxy proteins designated as Wx-A1, Wx-B1 and Wx-D1 with molecular mass ranging from 59 to 60 kDa, and encoded by three genes at the Wx-A1, Wx-B1 and Wx-D1 loci, located on chromosome arms 7AS, 4AL and 7DS, respectively (Preiss and Sivak, 1996). Several studies indicate that there is a strong correlation between the amylose content across cultivars and the activity of such Wx loci. Common wheat normally has the three waxy isoforms. By natural mutation, however, some genotypes may possess a null at one of the loci; the term partial waxy wheat was thus coined to identify such genotypes. So far no wheat line naturally devoid of all the three GBSS isoforms was identified in the international sampling of wheat. By conventional breeding practices some lines were selected carrying two or, in few cases, three null alleles. Only in case of three null alleles (waxy wheat), the amylose content falls below 3% (Van Hung et al., 2007). The Japanese researchers have been the first ones producing completely waxy wheat by using traditional hybridizations between Wx-D1 single null line BaiHuo and the Wx-A1/Wx-B1 double null line “Kanto 107” (Nakamura, 1995). Since 1995 numerous efforts to develop waxy wheat cultivars are underway in Europe, North America, Japan and Australia (Graybosh et al. 1998).

The starch retrogradation is believed to be the major cause of breadcrumb firming, commonly referred to as bread staling; in particular the amylose is assumed to be the main contributor to bread firming. Food industry is lately increasingly interested towards waxy (amylose-free) starch because its application to bakery products seems to be able to naturally induce retardation of staling, extended shelf-life (Van Hung et al., 2007).

Graybosh et al. (2003) investigated the influence of environmental conditions on waxy wheat productivity and quality and concluded that significant variations occurred for most of flour quality traits from wheat lines grown produced in different environments in the US. In particular significant variations were observed for seed weight, grain hardness, grain ash and protein content, SDS sedimentation volume, flour yield, flour ash and protein content.

Considering the high influence of the environments on wheat productivity and quality (Graybosh et al. 2003; Peterson et al. 1992,1998) it is hence unlikely that waxy wheat lines produced in other countries could be successfully cultivated in Italy. It is also worth mentioning that consumer resistance and existing regulations do not allow to employ genetically engineered foods. For these reasons waxy wheats obtained from traditional crossing starting from partial-waxy autochthonous landraces have to be employed.

In the perspective of developing waxy wheat lines suitable for cultivation in Italy, various research activities have been accomplished since ten years in this country (Boggini et al. 2001, Urbano et al. 2002, Monari et al. 2005, Caramanico et al. 2010).

Aim of the present work is the evaluation of agronomic and technological performances of a set of 18 waxy lines derived from a breeding program started in 2000 and set up at CRA-SCV (Italy) from about 60 partial-waxy cultivars previously identified from a screening of Italian germplasm, represented by about 300 cultivars.

2.2 MATERIALS AND METHODS

Materials

A set of 18 Italian waxy wheat lines (IWWL), was obtained by mating F1 offsprings from the cross between two partial-waxy cultivars identified in Italian germplasm, Cologna lunga (*Wx-D1*) and Barra (*Wx-B1*) with two homozygous waxy wheat kindly provided by C. Morris (Washington State Univ., Pullman, WA) (Morris & Konzak, 2001). These waxy wheats are: WQL6K107-BHWX14-7, PI 612546, hard type (indicated as BHWX14-7 in this study), and WQL6K107-BHWX2-2a, PI 612545, soft type (indicated as BHWX2-2a). The initial generations were grown in greenhouse; from F2S2 the plants were grown in the field (mini plot consisting of three rows of 1m), taking all the appropriate cares to avoid cross pollination. Details on IWWLs are reported in Tab.1.
Starting from the first selfing generations the IWWLs have been characterized by waxy phenotype by means of a colorimetric assay. All plants were harvested and a sample of 20 seeds/plant was analysed. The colorimetric analysis was performed with a solution of KI/I_2 (2% KI; 1%I_2) on the embryo-less half of the kernels. Non waxy and waxy starches show specific colors of blue-black and red-brown respectively upon iodine treatment (Nakamura et al.,1995). IWWL with more than 95% homozygosity were selected and further checked by means of electrophoretic analysis of seed storage proteins (Pogna et al.,1990; Pogna et al.,1989).

Table 1. Pedigree of Italian waxy wheat lines

Italian waxy wheat lines	pedigree
WX50 WX73 WX119 WX120	COLOGNA LUNGA / BARRA//WQL6K107-BHWX2-2a
WX39 WX70 WX71 WX74 WX80 WX83 WX104 WX107 WX116 WX118 WX123 WX124 WX125 WX128	COLOGNA LUNGA /BARRA//WQL6K107-BHWX14-7

Methods

Field experiments

During the growing seasons 2008-2009, 2009-2010 and 2010-2011 the 18 IWWLs were planted in Sant'Angelo Lodigiano, Lodi, Italy in 10 m^2 plots, following a completely randomized block design with two replications. Each line was sown in six-row plot (row length 1.50 m, spaced 0.30 m apart) at a density of 450 germinating caryopses m^{-2}. Standard agronomic practices were applied including seed dressing, chemical control of weeds, 130 kg ha^{-2} of nitrogen in two top-dressing. Fungicides and pesticides were not used. Two waxy wheat lines (WQL6K107-BHWX14-7, and WQL6K107-BHWX2-2a,) and the non waxy cv Aubusson (ordinary bread making wheat, wild type, wt) were grown in the same field as controls.
The following agronomic characteristics were recorded: grain yield (t ha^{-1}), plant height (cm), heading time (days after 1st April), resistance to powdery mildew (*Blumeria graminis*), to brown rust (*Puccinia recondita*) and to Septoria tritici blotch (*Septoria tritici*), frost damage (Borghi et al.,1986). Test weight (kg hL^{-1}) was evaluated, according to AACC 55-10 (AACC International, 2000); thousand kernel weight (g; TKW) was determined on three 100-kernel samples according to ISO 520.

Proximate analysis and rheological properties of flour

Samples of 50 g wheat kernels were ground to wholemeal with a 1 mm sieve Cyclotec mill (FossTecator AB, Höganäs, Sweden). Wholemeal protein content (N x 5.7 dry weight, AACC 39-10, AACC International, 2000) and hardness (AACC39-70A) were determined by the NIR System Model 6500 (Foss NIR Systems, Laurel, MD). The SDS sedimentation volume was determined according to Preston et al. (1982). The chemical proximate composition was determined on flour prepared by milling the grain with a Bona Quadrumat Labormill (Bona, Monza, Italy). The amylose content of flour was measured by enzymatic kit Megazyme International (Wicklow, Ireland Ltd.). Flour Gluten Index (AACC 38-12) was estimated by the Glutomatic 2200, Gluten Index Centrifuge 2015 and Glutork 2020 (Perten Instruments, Stockholm, Sweden) whereas flour Hagberg Falling Number (FN, s; Esetek Instr. Srl, Roma, Italy) was assessed according to ISO 3093. The rheological evaluation were performed by the Chopin alveograph (ICC 121-1992) and by a Brabender Farinograph (Brabender OHG, Duisburg, Germany) using a 50g mixer according to the ICC 115-D (ICC 1992). Materials used were produced during the growing season 2008-2009.

Bread-making procedure

Bread loaves from 25 g of flour were produced according to AACC10-10B with some modifications (e.g. in the absence of milk and potassium bromated) introduced to make the method more similar to the one traditionally used in Italy (Borghi et al., 1996). The baking formula (flour basis) was flour 50.0g (14%db), sugar 2.0 g, salt 1.0 g, shortening 1.5 g, yeast 2.0 g and ascorbic acid 2 ppm. The amount of water added to form optimum dough was calculated from the water absorption of the flour during a Farinograph test performed to obtain an optimal consistence of 500 BU. All ingredients were mixed for 3 min. The dough was divided into two halves (about 45 g/piece) and then fermented in cabinet at 30°C at a relative humidity of 80% for 150 min. Punching was done twice during the fermentation (after 50 min and after 75 min). All doughs were placed in apposite baking pans (4.2 x 7.0 cm in top, 3.1 x 6.0 cm in bottom, and 4.0 cm in depth) and fermented for 70 min before baking. Dough pieces were baked at 220°C for 20 min. Bread loaves were allowed to cool for a minimum of 60 min before further test. Bread volume was measured by rapeseed displacement. A commercial flour of high bread-making quality was used as control for all technological analyses. Materials used were produced during the growing season 2008-2009.
All the analyses were carried out in duplicate.

Statistical analyses

Least significant difference (l.s.d. p=0.05) was used to compare the mean values of the parameters considered within the waxy class. The analysis of variance was performed by using the Statgraphics Plus 4.0 and the Principal Components Analysis (PCA) was performed by using the Unscrambler® v.9.2 software (Camo A/S, Oslo, Norway). Agronomical data were computed by using the Agrobase Generation II sofware, v. 33.10.1 (2001-2010, Agronomix Software, Inc., Winnipeg, Canada).

2.3 RESULTS AND DISCUSSION

The results concerning grain yield and other agronomic traits derived from the three-years experimental trial are shown in Table 2. Grain yield of IWWLs ranged from 3.23 t·ha^{-1} (wx107) to 4.70 t·ha^{-1} (wx119) with a mean value of 4.15 t·ha^{-1}, higher, although not in a statistically significant way, than the corresponding value for the waxy standards (WQL6K107-BHWX14-7; WQL6K107-BHWX2-2a). Also the difference between IWWL and non waxy cultivar grain yield was not significant. This seems to confirm that a possible grain yield penalty cannot be associated with use of waxy trait in wheat breeding programs as reported in Graybosch et al. (2003).

All the IWWLs were relatively tall (from 73 cm to 95 cm) probably also in consequence of the fact that a parental wheat line was Cologna lunga characterized by an average plant height of more than 120 cm. This trait could make the waxy lines more susceptible to lodging.

As far as the growth cycle is concerned, IWWLs resulted on average early-flowering, the earliest lines (wx73, wx74, wx104, wx125) with a heading date of 31-32 days. Also the American standard resulted early-flowering with a heading date of 29-32 days.

The average value of test weight was high (78.6 kg·hL^{-1}), all IWWLs exceeding 75 kg·hL^{-1} which is the minimum value for wheat to be sold for bread-making, according to the Italian Synthetic Quality Index method (ISQ) (Borasio, 1997; Foca et al., 2007). These results, together with the values registered for thousand kernel weight (35.1 g on average), are indicators of good kernel filling levels.

The IWWLs mean protein content ranged from 14.9% to 17.0% and resulted quite high in comparison with the values registered for the non waxy cultivar Aubusson in agreement with Van Hung et al. (2006).

Table. 2- Agronomic characteristics of Italian waxy wheat lines. The mean values of thee groing seasons.

Entry		Grain yield ($t\ ha^{-1}$)	Plant height (cm)	Heading (days from Apr 1 st)	Test weight ($kg\ hL^{-1}$)	TKW (g)	Protein content (% d.m.)
wx39	wx	3.74	79	36	75.4	34.6	16.4
wx50	wx	3.68	81	34	78.4	31.8	16.8
wx70	wx	4.26	81	44	80.6	33.2	14.9
wx71	wx	3.98	76	38	78.8	32.6	15.0
wx73	wx	3.95	93	32	76.6	27.9	15.2
wx74	wx	3.74	73	31	78.8	37.0	16.6
wx80	wx	4.61	84	33	76.9	38.0	16.1
wx83	wx	4.67	82	37	81.0	34.8	15.0
wx104	wx	4.21	87	32	77.2	38.3	17.0
wx107	wx	3.23	74	34	77.9	37.5	15.9
wx116	wx	3.83	95	36	78.7	36.0	15.8
wx118	wx	4.65	78	33	78.6	38.9	15.7
wx119	wx	4.70	80	33	79.2	39.3	15.9
wx120	wx	4.56	85	38	81.1	33.7	15.1
wx123	wx	4.24	84	36	80.0	36.7	15.5
wx124	wx	4.42	89	41	79.7	34.5	16.1
wx125	wx	4.03	81	32	77.5	32.8	15.5
wx128	wx	4.25	89	41	78.6	33.6	15.3
Mean		**4.15**	**83**	**36**	**78.6**	**35.1**	**15.8**
LSD (P=0.05)		**1.17**	**12**	**9**	**3.1**	**4.2**	**1.6**
SE		**0.48**	**5**	**2**	**1.3**	**1.7**	**0.7**
BHWX14-7	wx	3.52	69	32	74.5	29.0	16.4
BHWX2-2a	wx	3.99	91	29	78.2	31.3	15.4
Aubusson	wt	4.92	68	44	70.4	30.7	12.4

Regarding disease resistance, the values reported in Table 3 relate only to two growing seasons, because IWWLs are early-flowering and during the third and last growing season the scarcity of rain during the short time elapsed before heading did not allow the development of diseases against which resistance had to be evaluated.

The lines wx70, wx71, wx116 and wx118, were particularly interesting because they resulted completely resistant to both powdery mildew and brown rust. Only wx83 seems to be as frost susceptible as Aubusson (wx83 is the line with the lowest level of frost damage).

During the last growing season a late sowing has also been accomplished. Data not shown suggest that all lines were quite suitable for late sowing. Performances resulted indeed comparable with the first autumn sow for average TKW (35.6 g) and heading (38 days) whereas the most penalized agronomic parameters were grain yield (2.27 t ha^{-1}) and test weight (73.8 $kg{\cdot}hL^{-1}$). This seems to be in agreement with conclusions reported in Sattar et al. (2010)

Table 3. Main disease resistance and frost damage of Italian waxy wheat lines. Diseaes index: 0= no desease, 9=severe desease. Frost damage index: 0= no damage, 9= severe damage

		Main diseases									Frost damage		
		Powdery mildew			Septoria	blotch		Brown rust			(Index)		
Lines		2009	2010	Mean	2009	2010	Mean	2009	2010	Mean	2009	2010	Mean
wx39	wx	3	3	3	8	4	6	3	2	3	7	5	6
wx50	wx	0	2	1	8	3	6	1	3	2	7	6	7
wx70	wx	0	2	1	5	3	4	2	1	1	8	7	8
wx71	wx	0	3	2	7	4	6	0	0	0	7	8	8
wx73	wx	4	2	3	6	3	5	0	4	2	8	6	7
wx74	wx	6	0	3	7	5	6	4	3	4	7	5	6
wx80	wx	1	1	1	7	1	4	3	0	2	7	9	8
wx83	wx	5	1	3	5	1	3	3	1	2	5	5	5
wx104	wx	0	2	1	6	3	5	3	2	3	6	6	6
wx107	wx	2	3	3	5	4	5	4	0	2	7	6	7
wx116	wx	0	1	1	7	1	4	0	1	1	7	5	6
wx118	wx	0	1	1	6	4	5	0	3	2	7	5	6
wx119	wx	2	0	1	7	5	6	4	2	3	7	7	7
wx120	wx	4	0	2	6	4	5	4	3	4	7	8	8
wx123	wx	4	3	4	6	4	5	3	1	2	7	7	7
wx124	wx	2	1	2	6	1	4	3	0	2	6	6	6
wx125	wx	5	1	3	7	1	4	3	1	2	6	7	7
wx128	wx	0	1	1	7	1	4	4	1	3	6	6	6
mean		**2**	**2**	**2**	**6**	**3**	**5**	**2**	**2**	**2**	**7**	**6**	**7**
SE		**0.5**	**0.2**	**0.2**	**0.2**	**0.4**	**0.2**	**0.4**	**0.3**	**0.2**	**0.2**	**0.3**	**0.2**
BHWX14-7	wx	0	4	2	8	1	4	0	0	0	8	7	8
BHWX2-2a	wx	0	0	0	8	1	4	0	0	0	7	5	6
Aubusson	wt	0	0	0	6	3	5	2	0	1	5	5	5

The IWWLs showed an average amylose content of 1.4±0.5% (Table 4) quite similar to that of the waxy standard ones and very significantly different from that of the cv Aubusson (26.9%) and commercial flour (27.4%). The value of FN (not shown) were in agreement with data in the literature. The average value of FN (65 ± 1 s) was much lower than that of the non-waxy control (581 s). Such a low value seems not to be related to the alpha-amylase activity but only dependent on waxy starch characteristics (Graybosch et al., 2000). The hardness index was also measured because it strongly affects damaged starch content, flour and grain water absorption and flour yield. Based on the index values above reported, out of the 18 Italian waxy lines, 3 were classified as soft, 6 medium and 9 hard grain.

The average milling yield of IWWLs has resulted 47.1% (hard), 48.3% (medium) and 53.0% (soft). These values are lower than the Aubusson yield, this being in agreement with Graybosch et al. (2003) and Yasui et al.(1999) who verified that waxy wheat flour yields are significantly lower than non WHW yields.

The IWWL protein content resulted on average quite high in agreement with Van Hung et al. (2006) and ranged from 12.3% to 17.2% in case of flours. For Wx70, Wx74, Wx107, Wx118 and Wx128 a high protein content was associated with high Gluten Index and SSV values indicating a good gluten quality. However only few lines have a good gluten quality because 66% of the waxy lines have SSV > 60mL and only 28% have gluten index over 70. This quality is nevertheless much higher than that of the two control waxy lines. For these samples the SSV has indeed resulted below 46 mL whereas the gluten index resulted 46 for BHWX14-7 and could not be measured for BHWX2-2a.

Table 4. Characteristics of Italian waxy and non-waxy flours.

Lines		Amylose content (%)	Proteins content (% d.m.)	Gluten Index	SSV (mL)	Hardness (Index)	Flour yield
wx39	wx	1.8	15.8	43	58	M	43.9
wx50	wx	2.1	17.2	45	56	M	47.5
wx70	wx	1.4	13.6	84	77	H	48.7
wx71	wx	1.0	12.3	74	74	S	51.3
wx73	wx	1.0	14.0	51	55	S	56.7
wx74	wx	1.5	16.1	44	55	H	47.0
wx80	wx	1.0	15.0	60	61	M	49.6
wx83	wx	2.6	15.5	58	63	M	49.2
wx104	wx	1.4	16.7	50	66	H	44.8
wx107	wx	1.0	14.8	85	72	H	47.7
wx116	wx	0.8	15.1	41	58	H	47.7
wx118	wx	1.4	14.1	72	73	S	51.1
wx119	wx	1.1	15.0	63	61	H	49.5
wx120	wx	1.4	15.3	43	63	H	46.6
wx123	wx	1.7	15.1	60	75	M	47.0
wx124	wx	0.7	13.5	43	66	M	52.3
wx125	wx	2.0	15.2	55	60	H	44.7
wx128	wx	1.7	14.4	70	76	H	47.4
mean		**1.4**	**14.9**	**56**	**64**		**48.5**
SE		**0.1**	**0.3**	**3**	**2**		**0.7**
BHWX14-7	wx	1.6	17.3	46	42	H	43.3
BHWX2-2a	wx	1.7	14.7	nd	46	S	49.2
Aubusson	wt	26.9	11.7	85	75	H	56.5
Commercial flour	wt	n.d.	13.8	88	80		n.d.

Technological characteristics of waxy wheat lines are reported in Table 5. Concerning the values of the alveograph W, if we consider a threshold level of 200 J*10^{-4} (which is the minimum value for eligibility to the above mentioned ISQ classification), for bread making quality classification, it can be seen that some waxy lines overcame this limit. Moreover, lines wx70, wx123 and wx128 had very high strength, comparable to the one registered for improver wheats (W>300 J*10^{-4}). Despite the high W value, all waxy wheat lines have required high pressure (P) but had very low extension (L) values and a consequently extremely high alveograph P/L value (P/L main 2.45). P/L values are usually positively correlated to the ability of wheat flours to retain gas during baking (Walker et al., 1996) but in this case indicate an excessive tenacity (and an in-elastic dough) that makes dough use for the baking test very difficult. The IWWL farinograph data showed in general high values for water absorption, ranging from 70.3% to 78.7% (Table 5) and were significantly higher than those of non-waxy wheat flour. According to Morita et al. (2002a), the high values registered for water absorption are due to a high amount of protein and dietary fiber. The high water absorption of waxy flour could explain waxy dough high tenacity, infact the amount of water needed to produce the dough used for the alveograph analysis is much lower than the water amount needed to obtain an optimal consistence of waxy dough (Caramanico et al, 2011; Garimella Purna, 2010). Despite the high protein content and in agreement with other authors (Hung et al. 2005; Morita et al. 2002 and Abdel-Aal et al. 2002) the dough made with waxy wheat flours exhibited lower stability during flour mixing. Only wx70, wx71, wx118 and wx123 had stability higher than 4 min, the minimum value for eligibility to ordinary bread making wheat according to ISQ classification. As a direct consequence, also the mechanical tolerance index was high (134 UB on average).

Previous studies described in the literature indicate that the bread made from WHW has usually slightly larger volume than bread from the non waxy wheat flour (Van Hung et al., 2006). In case of the Italian samples the low dough stability registered could have in principle determined low volume breads. Nevertheless Italian WHW lines produced quite good results. The height of Italian waxy breads resulted indeed 26% higher than that of the American breads on average. The bread volume of Italian waxy wheat lines ranged from 148 to 250mL and 74% of Italian waxy wheat lines had a bread specific volume higher than that of commercial flour (5.38 ±0.18mL/g).

Table 5. Bread making characteristics of Italian waxy wheat lines.

		Alveograph				**Farinograph**				**Baking Test**			
Lines		**W ($J\ 10^{-4}$)**	**P (mm)**	**L (mm)**	**P/L**	**Water Absorption (%)**	**Develop. Time (min)**	**Stability (min)**	**Soft. (UB)**	**Bread volume (mL)**	**Weight (g)**	**Height (mm)**	**Specific Volume (mL/g)**
wx39	wx	199	121	55	2.21	74.5	3.2	1.5	176	148	32.5	65	4.53
wx50	wx	232	118	67	1.78	75.0	4.3	2.7	157	215	33.4	80	6.43
wx70	wx	350	176	52	3.38	75.7	4.7	4.6	83	239	33.4	91	7.14
wx71	wx	291	187	38	4.99	71.7	4.0	4.8	77	183	34.3	79	5.32
wx73	wx	169	108	52	2.09	70.3	3.3	2.0	105	170	32.7	77	5.21
wx74	wx	181	108	55	1.98	77.9	3.6	1.5	182	221	32.4	86	6.84
wx80	wx	233	118	68	1.73	73.5	3.5	2.0	142	220	33.5	91	6.56
wx83	wx	230	142	43	3.29	74.6	3.2	2.6	140	191	34.0	83	5.63
wx104	wx	232	128	57	2.26	78.7	3.3	1.9	173	238	33.5	94	7.08
wx107	wx	265	128	67	1.93	74.6	3.6	3.6	121	213	34.1	88	6.24
wx116	wx	179	100	64	1.56	74.8	3.0	1.6	151	220	33.4	86	6.58
wx118	wx	316	171	51	3.39	73.9	3.5	4.2	118	220	34.0	87	6.48
wx119	wx	311	131	84	1.56	76.9	3.9	3.5	134	246	33.6	94	7.33
wx120	wx	122	86	47	1.85	74.0	3.5	1.5	180	150	34.7	67	4.32
wx123	wx	366	185	52	3.55	76.3	4.6	5.3	99	250	34.3	97	7.29
wx124	wx	272	160	44	3.68	72.6	3.3	3.1	124	219	34.6	85	6.32
wx125	wx	240	131	56	2.36	76.5	3.7	2.1	152	191	34.5	83	5.55
wx128	wx	345	160	63	2.54	75.4	4.0	3.8	99	231	34.1	93	6.78
Mean		**252**	**136**	**56**	**2.45**	**74.8**	**3.7**	**2.9**	**134**	**209**	**33.7**	**85**	**6.20**
SE		16	7	3	0.22	0.5	0.1	0.3	8	7	0.2	2	0.21
BHWX14-7	wx	166	106	56	1.89	74.4	2.7	0.9	191	148	33.5	62	4.40
BHWX2-2a	wx	135	106	36	2.99	70.9	2.8	1.0	171	153	31.9	63	4.78
Aubusson		155	55	98	0.56	52.5	1.8	4.3	63	131	32.6	67	4.03
Commercial flour		354	82	138	0.59	56.0	2.2	18.8	10	178	33.1	77	5.38

A PCA model was made including all waxy wheats and the two controls (20 samples in total) and 27 variables related to technological and agronomic parameters were considered in order to address differences and similarities between waxy samples (Fig. 1). The explained variances on PC1 and PC2 account for 52% of variance. As regards the PC1, the variability is related to parameters both associated to W alveograph, gluten index and SSV flour, P alveograph, bread weight, grain yield, test weight, heading and stability farinograph. As regards the PC2, the variability is related to parameters positively associated with grain hardness, bread volume and height, farinograph degree of softening, L alveograph, thousand kernel weight, brown rust, protein content, whereas frost damage, and flour yield are negatively associated. The score plot shows that the lines wx123, wx128, wx70, wx118, wx71, wx124 are positioned on the right in the plane area and have good technological characteristics. On the left in the plane there are the lines that don't have a good potential and two American waxy lines.

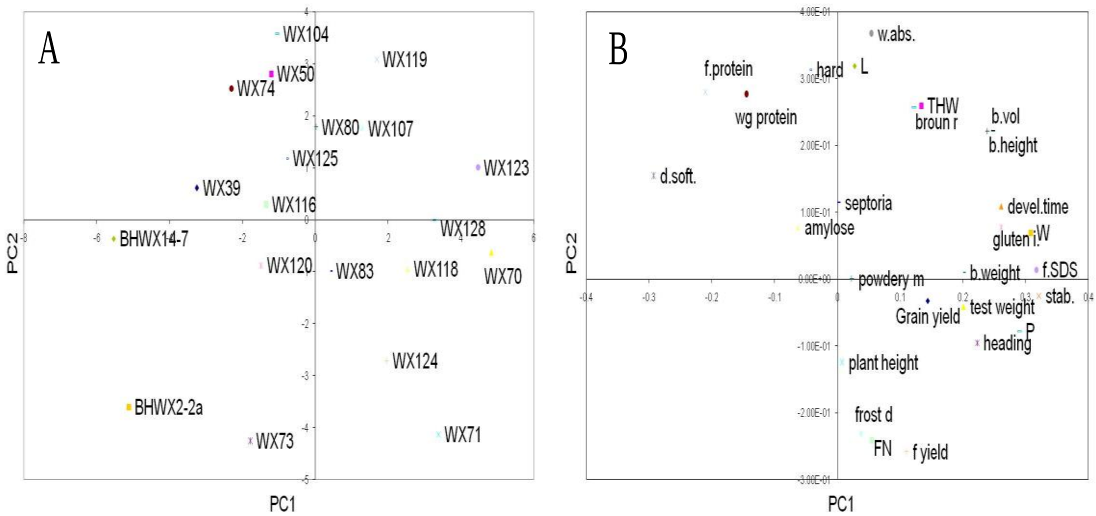

Figure 1. Score Plot (A) and Loading Plot (B) of the PCA performed on the WHW samples (PC1:32%; PC2: 20%)

2.4 CONCLUSIONS

Despite the supposed high influence of different environments on wheat productivity and quality, agronomical tests repeated during three years did not allow detecting significant differences between IWWL and American waxy as well as between IWWL and not waxy wheat in relation to their agronomical characteristics. Important differences related to technical

characteristics have been instead observed. Almost all IWWL showed higher bread making qualities with respect to the controls considered.
The IWWL wx123, wx128, wx70, wx118, wx71, wx124 showed particularly good performances. In particular these lines resulted characterized by higher values of technological parameters and of parameters related to gluten quality. These parameters are the gluten index, the SDS sedimentation volume, the farinograph stability and the bread specific volume. Some of these lines will be further tested during the next growing seasons in three different locations in order to confirm results achieved.

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3. PHYSICOCHEMICAL AND STRUCTURAL CHARACTERISTICS OF STARCH FROM ITALIAN WAXY WHEATS

3.1 STATE OF THE ART

Starch is the most abundant constituent in wheat flour and plays an extremely important role in their pasting properties. The differences in amylose/amylopectin ratios of starches result in different rheological properties of flour, which are involved in the texture and quality of end use products.

Starch is constituted of two polymers of glucose units: amylose that is essentially a linear chain of glucose units linked through α-D-(1-4) glycoside bonds, and amylopectin that is constituted of linear α-D-(1-4) –branches connected to each other through α-D-(1-6) glycoside bonds. The proportion of α-D-(1-6) bonds compared to α-D-(1-4) bonds in amylopectin is estimated between 5 and 95% (Tester et al., 2004). Variations also occur in the number, length and distribution of amylopectin branches, which depend on the species, cultivar and growing conditions (Buléon et al.,1998). The typical proportion of amylose and amylopectin in wheat is about 25% and 75% respectively, although variations occur depending on genetic and agronomic conditions. In case of waxy wheat the amylose content falls below 3% (Hung et al., 2007)

In its native form, amylopectin is a semi-crystalline macromolecule constituted of alternating crystalline and amorphous lamellae called clusters. In the crystalline regions, amylopectin linear branches form double helices arranged in parallel with each other (A chain), while the amorphous lamellae regroup the molecules branching points (B chain).

The crystalline order in starch granules is often the basic underlying factor influencing functional properties. Collapse of crystalline order within the starch granules manifests itself as irreversible changes in properties, such as granule swelling, pasting, loss of optical birefringence,, uncoiling and dissociation of the double helices, and starch solubility (Atweell et al., 1988; Singh et al., 2003). The order disorder transitions that occur on heating an aqueous suspension of starch granules have been extensively investigated using Differential Scanning Calorimetry (DSC) (Hayakawa et al.,1997, Yoo & Jane, 2002; Abdel-Aal et al., 2002). Starch transition temperature and gelatinization enthalpies by DSC may be related to characteristics of the starch granule, such as the degree of crystallinity. The waxy wheat starch shows only one peak for starch gelatinization, indicating no evidence of amylose-lypid complex. Gelatinization transition temperature (onset, peak and completion) and enthalpy variation (ΔH) of waxy wheat starch result significantly higher than those of non-waxy wheat starch. Waxy wheat starch also exhibits high resistance to retrogradation during storage. During this phase water molecules become bound to the crystalline clusters of starch double helices modifying the water mobility in the system. The DSC can be used for monitoring this process. The retrogradation enthalpy variation is lower than the gelatinization enthalpy, as retrograding starch does not form the same highly organized structure as granular starch.

Starch pasting behavior in aqueous systems depends on physical and chemical characteristics of starch granules, such as mean granule size, granule size distribution, amylose/amylopectin ratio. When starch molecules are heated in excess water, the crystalline structure is disrupted and water molecules are linked by hydrogen bonding to the exposed hydroxyl group of amylose and amylopectin: this phenomenon causes an increase in granule swelling_and solubility. In starch wheat, granules may swell up to 30 times their original volume without disintegration (Singh et al., 2003). It has been suggested that amylose plays a role in restricting initial swelling because this form of swelling proceeds more rapidly after amylose has firstly been exuded. The increase in starch solubility, with the concomitant increase in suspension clarity, is seen mainly as the result of granule swelling, permitting the exudation of the amylose. The extent of leaching of soluble mainly depends on the lipid content of the starch and the ability of the starch to form amylose-lipid complexes, that are insoluble in water and require higher temperature to dissociate (Morrison, 1988; Raphaelides & Karlakas, 1988). A reduced level of amylose also is associated with high values of swelling power in wheat starch (Wang & Seib, 1996; Sasaki & Matsuki, 1998), particularly at higher temperature as 90°C (Abdel-Aal et al., 2002).

Gelatinization and retrogradation phenomena have been widely studied by empiric rheological tests, as the Rapid Visco Analyzer (RVA) test (Hung et al., 2006; Hung et al. 2007; Qin et al. 2009; Bhattacharya et al. 2002; Graybosch 1998; Kiribuchi-Otobe et al. 1997, Lan et al. 2008, Yoo & Jane, 2002). In general these studies showed that in waxy wheat starch gelatinizes quickly and has a higher peak viscosity, higher breakdown, lower setback and lower peak temperature with respect to non-waxy starch.

This study aimed to investigate some of the main starch physicochemical and structural characteristics. In particular, the analyses have focused on thermal and pasting properties of flour from some waxy wheat lines adapted to the Italian environmental conditions. In order to highlight possible peculiarities of waxy wheat lines cultivated in Italy two American waxy wheat varieties and one non waxy wheat cultivar are taken as standard.

3.2 MATERIALS AND METHODS

Materials

Seven Italian waxy wheat lines (IWWL) were used for the analysis performed, out of which 4 were of hard type (wx70, wx104, wx107, wx128), 2 were of soft type (wx71 and wx118) and 1 of medium type (wx123). Two homozygous waxy wheat WQL6K107-BHWX14-7, PI 612546, hard type (indicated as BHWX14-7 in this study), and WQL6K107-BHWX2-2a, PI 612545, soft type (indicated as BHWX2-2a in this study), kindly provided by C. Morris (Washington State Univ., Pullman, WA) (Morris & Konzak, 2001) were used as control in all technological analyses together with the non-waxy cv Aubusson (ordinary bread making quality, wild type, wt) and a commercial flour of high bread-making quality.

Methods

Analysis of flours

The wheat grains were milled into flours on a Bona Quadrumat Labormill (Bona, Monza, Italy). Total and damaged starch content were determined enzymatically using the “Total Starch assay Kit (AACC76-13,2001) and the “ Starch Damage Assay Kit” (AACC 76-31, 2001) respectively (Megazyme International Ireland Ltd., Bray Business park, Bray, Co. Wicklow, Ireland).
Alpha-amylase activity of flours was determined by AACC 22-02 (AACC International, 2000) using a Megazyme assay kit. Enzyme activity was reported in Cerealpha Units (CU), where one CU is defined as the amount of enzyme, in the presence of excess of thermostable amyloglucosidase, required to release one micromole of *p*-nitrophenol from end blocked *p*-

nitrophenyl maltoheptaoside in one minute under the defined assay conditions**.** Arabinoxylan content, expressed as D- xylose, was determined by using phloroglucinol colorimetric reagent (Douglas, 1981). Finally flour Falling Number (FN, s; Esetek Instr. Srl, Roma, Italy) was assessed according to ISO 3093. All the analyses were carried out in quadruplicate.

Starch isolation

Starch was extracted from flour by using the method proposed by Batey et al. (1997) with minor modifications. Wheat starch was washed from flour by employing a Glutomatic Model 2220 gluten washer (Perten Instruments, Stockholm, Sweden) using 2x10 g samples of flour simultaneously. Starch was subjected to repeated water-washing, centrifugation and separation. Two of such washing cycles were applied by using 200 mL of distilled water for each wash. After each cycle the protein and tailing starch layer was carefully removed by scraping it of the sedimented starch. Residual pellet was further washed with pure methanol for at least four hours and starch was then separated by centrifugation.and finally dried at about 40 °C for at least 24 hours within a ventilated oven.

Pasting properties

Flour pasting properties were determined by using the Rapid Visco Analyzer test (RVA-4 model, Newport Scientific, Sidney, Australia), according to ICC162. Flour amounts of 3.5 g were dispersed in 25 mL of distilled water, scaling both sample and water weight on a 14% flour moisture basis. The suspension was subjected to the following temperature profile: holding the sample at 50°C for 1 min followed by heating the sample from 50 to 95°C in 3.75 min, then at 95°C for 2.5 min, cooling the sample back to 50°C in 3.75 min, and holding the sample at 50°C for 2 min. The following indices were considered: pasting temperature (PT, °C at which an initial increase in viscosity occurs), peak viscosity (PV, Pa·s; maximum paste viscosity achieved during the heating cycle), breakdown (BD, Pa·s, index of viscosity decrease during the first holding period, corresponding to the peak viscosity minus the viscosity after the holding period at 95°C), final viscosity (FV, Pa·s; paste viscosity achieved at the end of the cooling cycle), setback (SB, Pa·s; index of the viscosity increase during cooling, corresponding to the difference between the final viscosity and the viscosity reached after the first holding period) using Thermocline for Windows 3.6 (TCW3) software provided with the RVA. Measurements were performed in triplicate and the average value was used.

Thermal properties

Thermal characteristics of dough from waxy and non-waxy flour were determined by using differential scanning calorimetry (DSC) with a Perkin-Elmer DSC apparatus (model DSC 6, Waltham, Massachusetts, USA). A PerkinElmer DSC-6 with 60 µL sealed cells was used. The reference cell contained a suitable amount of distilled water. Measures were carried out in the 20–150°C range with 2.0°C/min scanning rate. Indium was used for calibration. The typical dough mass was 30 mg. Dough samples were manually mixed for 10 min before being analysed. The raw data were worked out with the dedicated software IFESTOS.

The base-line chosen to work out a given DSC trace was the DSC record of the immediate re-heating run. It was subtracted from the record of first DSC heating run, which corresponds to the apparent heat capacity $C_p(T)$ of the sample (per gram of dry matter), to obtain the trend of the excess heat capacity, C_p^{ex} (T), which allowed evaluation of the enthalpy drop ΔH by a straightforward integration of the corresponding trace (Fessas et al., 2008).

The behavior of water within wheat flour dough was inspected by a thermogravimetric (TG) analysis. The TG instrument was a Setaram TG-DSC111 (Lyon, France) with the simultaneous output of the thermal effect (heat flow *vs*. T), TG trace (mass loss *vs*. T) and its time derivative

(DTG). The typical sample mass was 30 mg. Dough samples were manually mixed for 10 min and were analysed after two hours of rest at room temperature in order to favor relaxation. Each run was repeated at least twice. The ratio between the heat flow and the related mass loss rate was found equal to the enthalpy of water evaporation throughout the investigated temperature range. This check confirmed that the mass loss was substantially related to water evaporation only, losses of other volatiles beingpoorly relevant. The DTG and heat flow traces were accordingly given in mgK^{-1} and $J\ K^{-1}g^{-1}$ units (with reference to the scanning rate used), respectively, the heat flow being converted into apparent specific heat, *C*p, dividing the instrument output (in mW units) by the product [sample mass·scanning rate] (in $mg\ K\ s^{-1}$ units) (Fessas et al., 2008).

Gel texture analysis

After RVA cycle, the stirring paddle was discarded and the flour paste was split into three small parts (7.0 g of gel each) enclosed into as many vessels kept at 4°C for two weeks.

The gel formed was subjected to texture profile analysis using a Texture Analyzer TA-HD- plus (Stable Micro System Ltd, UK) on days 0, 1, 3, 6 and 14. The RVA gel firmness was assessed based on the maximum force required to penetrate the gel with a cylindrical probe (4 mm diameter) up to 30% of gel height.

Mixolab test

Dough rheological properties were investigated by using mixolab (Chopin, Triplette et Renaud, Paris, France); this instrument allows mixing and simultaneous heating or cooling of the dough under controlled temperature. Required amount of flour for analysis was calculated by Mixolab software according to input values of flour moisture and water absorption. The water absorption was calculated as the water absorption of flour obtained, during Farinograph test, at a optimal consistence of 500 BU. The setting used in the test were 20 minutes for mixing at 30°C, temperature increase at 4°C/min until 90°C, 7 minutes holding at 90°C, temperature decrease at 4°C/min until 50°C and 5 minutes holding at 50°C. The mixing speed during the entire assay was 80 rpm. All the measurements were performed in duplicate and the average value was used.

Light Microscopy

Starch samples (10.0 mg each) isolated from waxy and not waxy wheat were suspended in 1.0 mL of Lugol's solution (Hinchman, 1973) and directly examined. Flowers from waxy and not waxy wheat were stained with 0.1% Toluidine blue in water and examined after 15 min and 75 min at 25°C. All examinations were carried out with an Olympus BX50 light microscope (Olympus, Tokyo, Japan), equipped with differential interference contrast (DIC) Nomasky and epipolarization filters. Images were captured with a Retiga 2000R camera.

Statistical analyses

The analysis of variance was performed by using the Statgraphics Plus 4.0. Least Significant Difference (l.s.d. p=0.05) was used to compare the mean values of the parameters considered.

3.3 RESULTS AND DISCUSSION

Physicochemical characteristics of starches

Starch, the major component in the wheat (*Triticum aestivum L*) kernel, affects quality and staling properties of wheat-based products. Table 1 indicates that the starch content of the IWWL flours ranged between about 77 and 81 % db and was comparable to that of the American waxy wheats and the non-waxy wheat samples. The amylose content was instead comparable and below 1.7% d.b in all waxy flours which resulted having a much lower amylose content with respect to non-waxy flour as expected. Data concerning damaged starch indicated that the ratio between damaged and total starch content was below 7% (corresponding to about 5 % db in Table 1) in case of starches from IWWL of soft type (i.e. wx71 and wx118) and was comparable to that of non-waxy starches of hard type. The corresponding ratios were higher for starches from IWWL of hard and medium type which achieved ratios values ranging between 10 and 14% (corresponding to 8-11 % db in Table 1). Considering that non-waxy starche of soft type has typically lower ratios of damaged starch with respect to non-waxy starches of hard type, these results indicate that waxy wheat starches had generally higher susceptibility to mechanical damage (e.g. during milling), in agreement with results found in the literature (Bettge et al., 2000 and Abdel-Aal et al., 2002). Table 1 shows Falling Number (FN) values around 65 s for waxy flour samples and around 460 s for non-waxy flour. Similar results for FN index are reported in Graybosh et al., 2000 and in Abdel-Aal et al., 2002. As showed by Table 1, FN values resulted completely uncorrelated to α-amylase activity for waxy flours, confirming that, contrary to what happens in case of non-waxy flours, the FN test cannot be applied to waxy flours as an indirect measure of α-amylase activity (Abdel-Aal et al. 2002). The values of α-amylase activity considered ranged between 0.05 and 0.30 CU, indicating that that wheat samples were sound and not germinated (Kim et al., 2002).

Table 1. Physicochemical characteristics of starches from flour samples considered in the study.

Lines		Amylose content (% d.b.)	Starch content (% d.b.)	Damaged starch (% d.b.)	α- amylase activity ($CU/g_{d.b.}$)	Falling number (s)
wx70	wx	1.5 ±0.4 a	79.4 ±2.2 cd	9.09 ±0.32 e	0.053±0.002 a	65 ±1 a
wx71	wx	1.0 ±0.4 a	80.6 ±0.9 d	5.34 ±0.27 a	0.143±0.003 c	66 ±1 a
wx104	wx	1.0 ±0.2 a	76.4 ±0.9 ab	10.76 ±0.60 f	0.069±0.001 b	62 ±1 a
wx107	wx	1.0 ±0.4 a	76.6 ±1.2 a	8.05 ±0.46 d	0.300±0.009 f	62 ±1 a
wx118	wx	1.4 ±0.1 a	79.5 ±0.8 cd	5.05 ±0.06 a	0.174±0.004 d	63 ±1 a
wx123	wx	1.4 ±0.5 a	79.2 ±0.6 bcd	7.79 ±0.40 cd	0.055±0.003 a	65 ±1 a
wx128	wx	1.7 ±0.3 a	78.1 ±1.2 a-d	7.24 ±0.35 c	0.137±0.004 c	66 ±1 a
BHWX14-7	wx	1.6 ±0.1 a	76.4 ±1.2 a	8.84 ±0.43 e	0.224±0.005 e	62 ±1 a
BHWX2-2a	wx	1.7 ±0.2 a	76.1 ±2.2 abc	5.56 ±0.65 b	0.050 ±0.002 a	66 ±1 a
Aubusson cv	wt	23.9 ±0.3 b	80.6 ±1.5 d	6.05 ±0.44 b	0.065±0.003 b	458 ±12 b

Values followed by same letter in the same column are not significantly different (P<0.05).

Interferential contrast microscopy allowed observing two types of starch granules, the large disk-shaped (type A) and the small sphere-shaped granules (type B), in both waxy and non-waxy samples (Figure 1). However type A starch granules seemed to prevail in non-waxy wheat and type B starch granules seemed to be in majority in waxy wheat. Moreover waxy wheat starch granules presented deformities and small cracks. Lugol staining showed that non-waxy starch granules became colored indicating amylose contents, whereas waxy starch granules did not generally take color. The mean granule size of type-A and type-B starch granules as measured by image analysis is reported in Table 2.

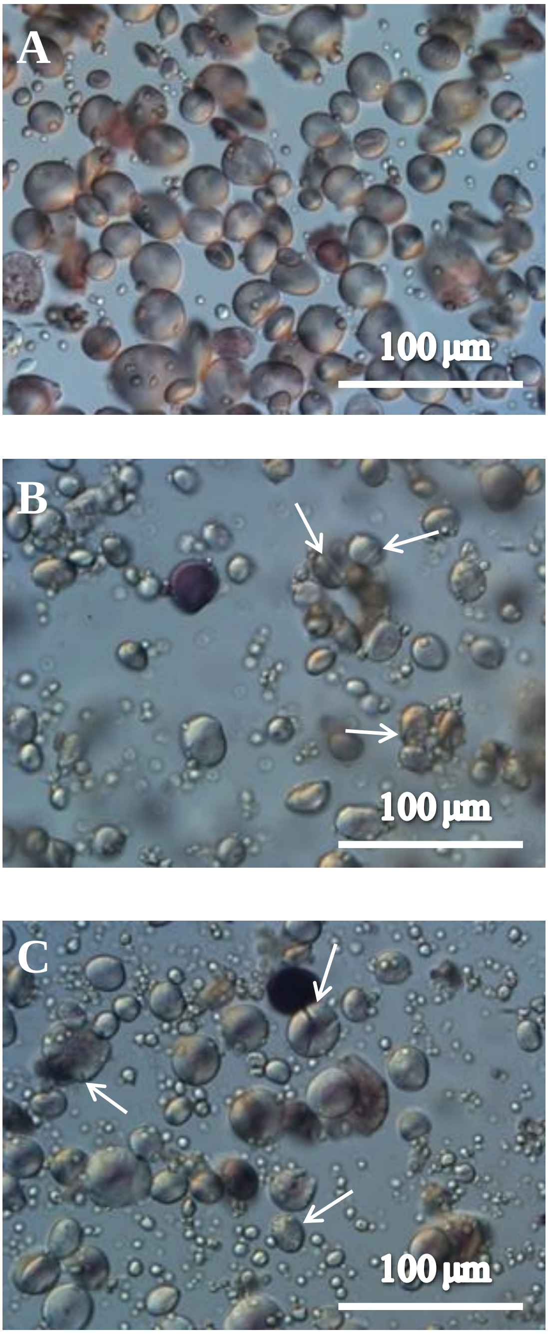

Figure 1. Images stained with Lugol's solution of starch granules of non-waxy wheat (A), BHWX2-2a (B) and wx118 (C) observed by interferential contrast microscopy. Arrows indicate granules with cracks

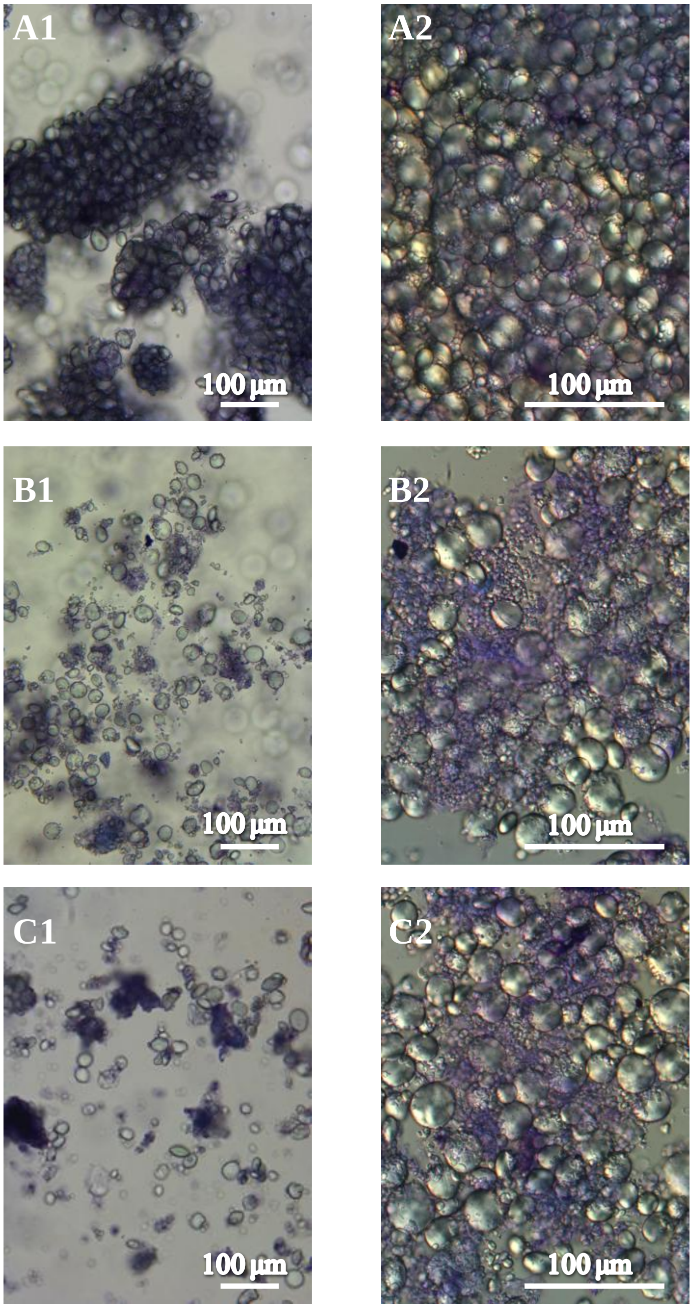

Figure 2. Images of flours from non-waxy wheat (A1, A2), BHWX2-2a (B1, B2) and wx118 (C1, C2) observed by interferential contrast microscopy. Flour samples were stained with Toluidine Blue for 10 minutes (left panel) and for 75 minutes (right panel) to highlight the protein component.

Table 2. Mean granule size[a] of type-A and type-B starch granules as measured by Image Analysis

samples		type A- starch granules (μm)	type B- starch granules (μm)
wx118	wx	11.2 ±1.7	2.1 ±0.2
BHWX2-2a	wx	9.2 ±1.6	2.9 ±0.5
Aubusson cv	wt	11.7 ±1.3	2.5 ±0.5

[a] Average over about 20 measurements ± standard deviation

The same microscopic analysis carried out on flours treated with Toluidine blue to stain proteins allowed observing that flour from non-waxy wheats had large size agglomerates made of starches kept together by clearly distinguishable proteins, whereas waxy flours had starch granules that appear dispersed with few and small agglomerates kept together by proteins (see pictures on the left side of Figure 2). After 75 minutes of staining, as a result of water imbibitions, the small starch granules were quite uniformly distributed around large starch granules in non-waxy flour whereas masses of small starch granules appeared randomly distributed among large starch granules in case of waxy flours. This different distribution after imbibitions could be related to a the different behavior of the dough from waxy wheat, as discussed in the following sections.

Pasting properties

Starch and flour pasting characteristics were studied by RVA analysis.

Results related to flours considered are reported in Table 3 below and show that waxy and non waxy samples did not differ substantially in terms of pasting temperatures. On the contrary, differences are relevant at temperatures higher than 70 °C. Viscosity of IWWL flour samples was indeed on average about 8 times higher than that of non-waxy flours already at 75 °C. Peak viscosity values were highly variable for IWWL flours (2077-2925·10^{-3} Pa·s) and were observed about 2 minutes earlier at a temperature around 80 °C and about 15 ° C lower than that registered for non-waxy flours peak viscosity. These differences could in principle be caused by the lack of amylose in waxy starch and by the higher percentage of smaller size granules and damaged granules observed in waxy starch which favor a faster hydration and swelling of this starch (Abdel-Aal et al., 2002) The subsequent viscosity decrease is due to starch granule breaking and was generally more pronounced in case of waxy samples (see breakdown values reported in Table 3) because of the absence of amylose. In case of non-waxy samples the presence of amylose trickling out in the slurry is indeed supposed to keep viscosity at higher levels during the whole heating phase.

Viscosity increased again after achieving a minimum value during the cooling phase, as indicated by the setback values reported in Table 3. It is well known that setback values give information about amylose re-organization during cooling (Hayakawa et al., 1997). Final viscosity and setback values were clearly lower in case of waxy flours. These results indicate that waxy wheat starch gelatinizes quickly and it is more resistant to retrogradation (Hung et al., 2006).

Table 3. RVA properties of waxy and non-waxy flour samples[a]

Lines		Pasting temperature (°C)		Viscosity at 75°C (10^{-3} Pa s)		PeakViscosity (10^{-3} Pa s)		Peak temperature (°C)		Breakdoun (10^{-3} Pa s)		Final Viscosity (10^{-3} Pa s)		Setback (10^{-3} Pa s)	
wx70	wx	66.2±0.7	a-d	1676 ±55	h	2390 ±20	d	79.0 ±0.5	bc	1435 ±33	e	1281±10	b	326 ±18	b
wx71	wx	67.1±0.1	de	2457 ±21	l	2925 ±27	f	77.2±0.1	a	1843 ±18	f	1457±13	d	376 ±1	de
wx104	wx	66.1±0.7	a-d	1346 ±20	e	2234 ±29	b	81.4±1.0	ef	1323 ±29	b	1343±9	c	342 ±8	bc
wx107	wx	65.8±0.6	abc	1836 ±13	i	2470 ±18	e	79.6 ±0.1	c	1465 ±9	e	1351±17	c	346 ±1	bcd
wx118	wx	66.7±0.4	b-e	1617 ±26	g	2377 ±2	d	78.3 ±0.5	b	1419 ±6	de	1341±8	c	383 ±7	e
wx123	wx	66.7±0.2	b-e	1270 ±3	d	2077 ±36	a	79.9 ±0.5	cd	1238±28	bc	1111±10	a	272 ±13	a
wx128	wx	65.7±0.7	ab	1450±3	f	2411 ±32	d	80.7 ±0.5	de	1388 ±37	de	1351±4	c	327 ±10	b
BHWX14-7	wx	66.0±0.8	a	1221 ±14	c	2310 ±19	c	82.0 ±0.1	f	1283 ±27	c	1364±9	c	337 ±11	bc
BHWX2-2a	wx	67.0±0.1	cde	1237±22	cd	2398 ±24	d	81.2±0.1	ef	1384±17	de	1383 ±6	c	369 ±2	cbe
Aubusson cv	wt	67.6±0.3	e	220 ±14	b	3119±31	g	95.0 ±0.1	g	1293 ±50	bc	3197±24	f	1371 ±40	f
Comm. Flour	wt	65.8±0.6	ab	181 ±3	a	2257±15	b	95.0±0.1	g	1105 ±6	a	2495±67	e	1344 ±48	f

Average over 3 measurements ± standard deviation. Values followed by same letter in the same column are not significantly different (P<0.05).

It has been demonstrated that the peak viscosity of flours is related to α-amylase activity. In particular some authors (Yasui et al., 1999; Lan et al., 2008; Garimella Purna, 2010) observed that this parameter markedly increases when the α-amylase activity is inhibited. This might in principle indicate that peak viscosity extent is negatively correlated to α-amylase activity in waxy wheat flours.

Nevertheless α-amylase activities of IWWL reported in Table 1 do not seem to be correlated to peak viscosity. This however might be due to the fact that the high heating rate applied in this analysis did not allow the action of the endogenous α-amylase (Mariotti et al., 2005).

Examples of RVA profiles obtained for starch extracted from IWWL flours and non-waxy flours are reported in Figure 3 and compared to the corresponding fours.

Starches from IWWL samples resulted having higher peak viscosity, higher breakdown, lower setback and an about 10 °C lower peak temperature with respect to non-waxy starch, in agreement with literature data (Qin et al. 2009, Bhattacharya et al. 2002, Hung et al. 2007, Graybosch 1998 and Kiribuchi-Otobe et al. 1997, Lan et al. 2008, Yoo & Jane, 2002, Hayakawa et al., 1997; Reddy & Sieb, 1999). The lower setback value of waxy starch is related to the lack of amylose. Waxy samples resulted indeed unable to develop a continuous gel matrix because they lacking of amylose, responsible for this structure (Hayakawa et al, 1997). It may be interesting to notice that setback values of waxy flours were slightly higher than setback values of related waxy starch in Figure 3: this slight difference could in principle be attributable to the presence of denatured proteins in waxy flours. In case of flour and starch from non-waxy wheat the breakdown values resulted comparable to the setback ones.

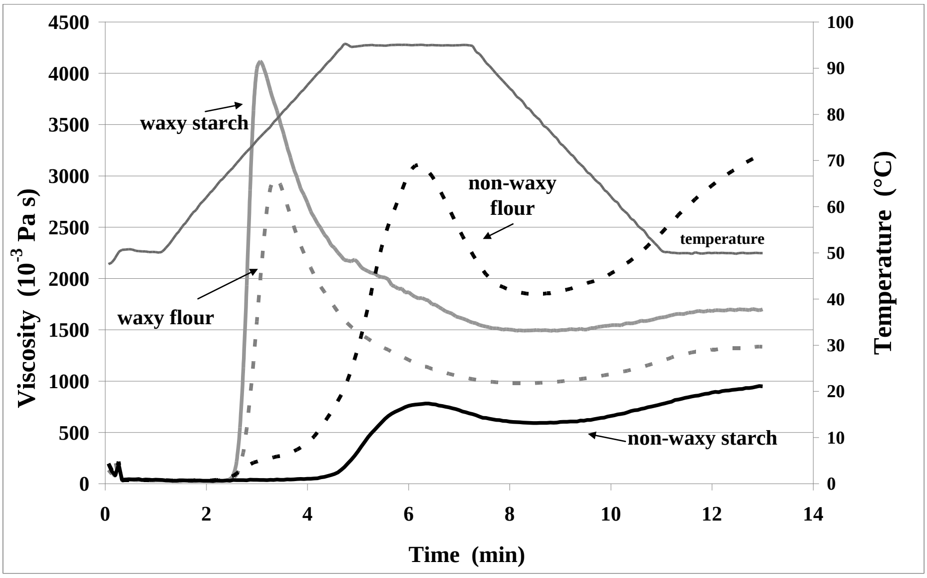

Figure 3. RVA profiles of flours (dotted line) and starch (continuous line) from Italian waxy and Aubusson cv (non-waxy) wheat.

Gel texture analysis

The texture of gels after storage is shown in Figure 4. As expected, the IWWL gel firmness did not increase during storage at 4 °C and achieved about $2.7 \cdot 10^{-2}$ N after 6 days of storage. On the contrary, firmness of gels from Aubusson cv and commercial flour increased dramatically during storage at 4 °C and resulted respectively about 23 and 16 times higher than IWWL gel firmness after 6 days of storage.

The compression of the gels can give information on amylopectin retrogradation. As well known, amylose reorganization is quick, reaching its maximum within a few hours following gelatinization (Goodfellow & Wilson, 1990). Amylopectin retrogradation takes instead place over several days, due to the higher molecular weight and structural complexity. The type of starch, in particular the amylose/amylopectin ratio, determines the texture of the gel: high amylose content starch (e.g starch from non-waxy wheats) give hard gels that break easily, while low amylose content starch (e.g. starch from waxy wheats) gives softer and sticky gels after about twelve hours (Bhattacharya et al., 2002).

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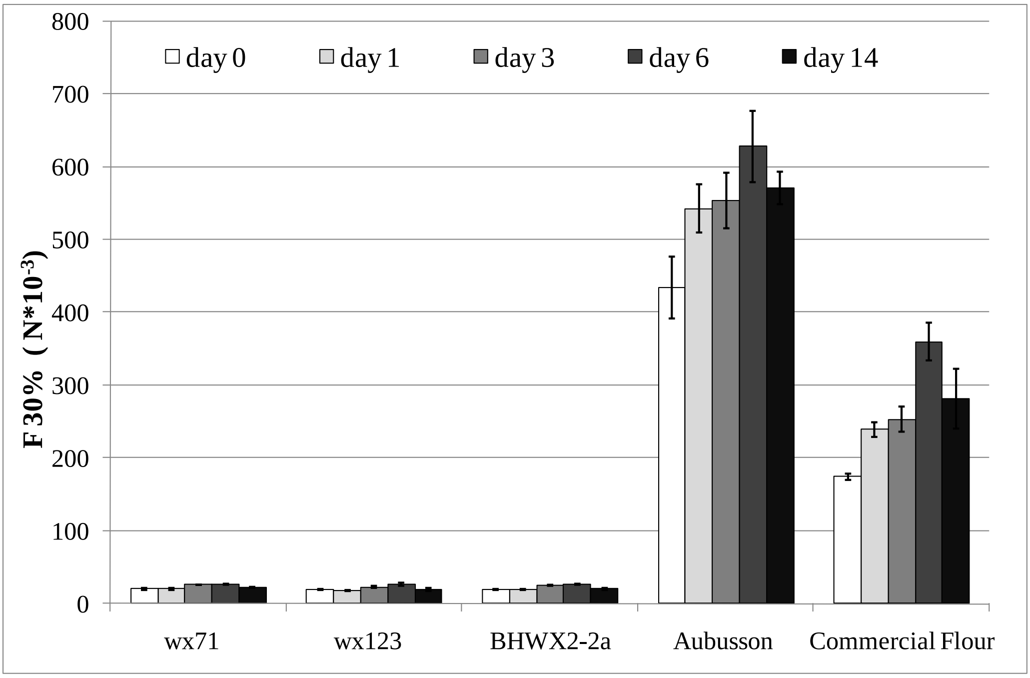

Figure 4. Firmness of RVA gels of flours from waxy flour and non waxy wheat during storage at 4 °C.

Mixolab test

In order to evaluate the extent of starch gelatinization and retrogradation phenomena in the moisture conditions of a real dough (and, therefore, obtain indication related to the baking process) dough was subjected to a dual mechanical shear stress and temperature constraint using the Mixolab device. In fact, while the RVA test measured the pasting properties of a suspension of flour in water (14:100), the Mixolab test measured the changes in consistency of a dough (flour:water = 100:60 on average). In particular, the absorptions applied were the same used for the farinograph test and ranged between 68-74% and 52-56% respectively in case of dough from waxy and non-waxy wheats.

Mixolab profiles reflect both starch and protein behavior under combined stresses of mixing and temperature cycles (heating and cooling under prefixed conditions) and relate in particular to protein weakening, starch gelatinization and starch gelling (Rosell et al. 2009).

Data concerning waxy dough stability registered during the first 20 minutes of mixing at 30 °C (Figure 5) were consistent with data provided by the farinograph. During the mixing at room temperature dough consistency decreased in a few minutes for waxy flours whereas it exhibited a lower decrease rate in case of non-waxy Aubusson flour and commercial non-waxy flour remained practically unchanged.Different behaviors are probably to be attributed to the different strengths of dough samples already highlighted by the stability values registered with the farinograph test.

During the subsequent heating phase the waxy dough consistency decreased and achieved a minimum value (between 0.17-0.22 Nm) at a higher dough temperature (60-62 °C) compared to dough from non-waxy flour which achieved a minimum torque value (between 0.38-0.47 Nm) at 53-57 °C. It was not possible to highlight any significant difference in the behavior of the various IWWL dough samples analysed, whereas the dough samples from American waxy flour seemed to be characterized by a lower consistency. In general the decreasing of dough consistency during the heating phase is associated to the weakening of the protein structure due to the effects of temperature increase and the mechanical stresses associated with mixing (Rosell & Collar, 2009).

Figure 5 also shows that waxy dough consistency achieved a maximum (0.5-0.48 Nm after 36 min) during the heating phase at a dough temperature of about 75 °C and that this maximum was significantly lower than the corresponding non-waxy wheat dough consistency (1.95 Nm) because of the lack of amylose during gelitinazation. The dough from wx71 flour seemed having higher consistency values with respect to other IWWL dough samples during this phase. It may be worth noticing that the wx71 sample resulted characterized by higher viscosity values with respect to the other IWWL samples during the RVA analysis.

After achieving its maximum, waxy dough consistency remained almost unchanged and increased again during the subsequent cooling phase, reaching values between 0.5 and 1.0 Nm. During the heating and the subsequent cooling phase non-waxy wheat dough consistency steadily increased markedly changing after 36 min of mixing and at cooling phase initiation. The dough consistency increase during the cooling phase is due to the gelation process of the starch. During this phase the re-association between the starch molecules is related to the retrogradation and reordering of the starch molecules themselves. These phenomena were more relevant in case of non-waxy wheat dough which achieved final consistency values about 4 times higher than those registered for waxy wheats. The dough from wx71 presented the highest final consistency value among dough samples from IWWL. It may be worth mentioning that the highest gelatinization intensity was reached at about 75 °C (corresponding to the maximum of consistency reached during the heating phase) in case of waxy dough and at a slightly lower temperature (corresponding to point at which the dough consistency increase rate changed markedly) in case of non-waxy dough.

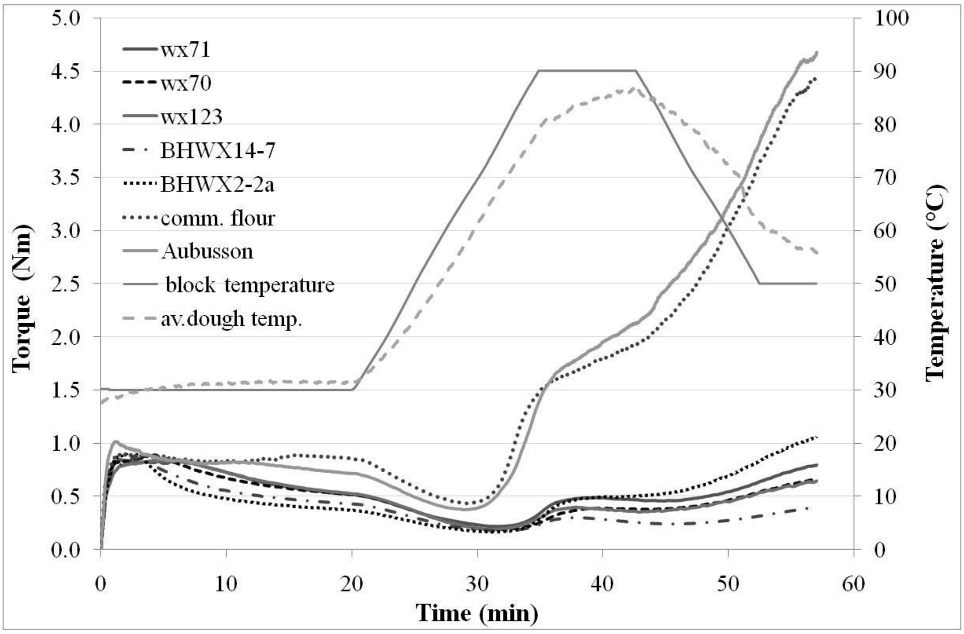

Figure 5. Mixolab profiles of dough samples from waxy wheat and non waxy wheat.

Thermal properties

The gelatinization phenomenon is an endothermic event which can be recorded by differential scanning calorimetry (DSC). This technique is used to detect phase changes, such as melting or transitions from one crystalline form to another, during which heat is either absorbed or released. The heat gained or lost during such transitions is determined by measuring the differential heat flow required to maintain both the starch sample and the inert reference at the same temperature, during heating at a constant rate. The width of the peaks due to gelatinization observed in DSC traces is related to granule size distribution of the samples, a wider peak indicating less homogeneity in granule sizes. The surface under these peaks, calculated by integration and normalized with the weight of the sample, gives the enthalpy variation (ΔH) of the gelatinization, in other words the energy required to gelatinize the sample. The starch with a low amylose content tends to have higher gelatinization enthalpy (Fujita et al.,1998, Hayakawa et al., 1997; Yasui et al., 1996).

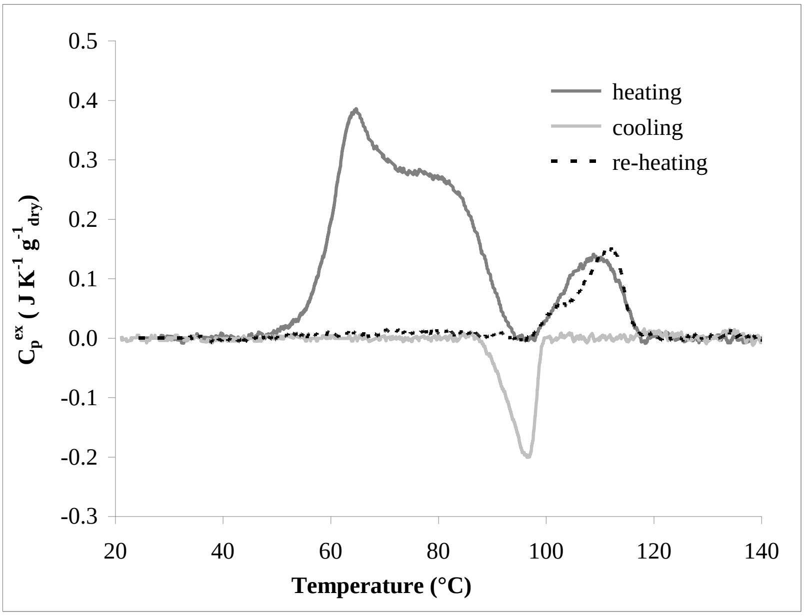

Figure 6. DSC traces of dough from Aubusson flour with 37% moisture content.

Figure 6 shows the typical DSC traces observed for a dough from non-waxy wheat. During heating the first peak around 65 °C corresponds to the first phase of dough gelatinization consisting in a partial hydration of starch granules. Gelatinization was then completed with the second peak observed at temperatures around 85 °C, whereas the third peak at temperatures around 110-115 °C has to be attributed to the dissociation of amylose-lipid complexes. The typical exothermic effect due to retrogradation showed up when the dough was cooled with the negative peak around 90 °C. DSC traces confirm that starch gelatinization is an irreversible phenomenon in the time period considered for this analysis. Indeed the peaks due to gelatinization registered during heating were no more detectable when the dough was re-heated.

DSC traces can be monitored for different values of dough moisture content in order to assess the influence of water on starch gelatinization. Figure 7 reports DSC traces registered in case of dough from wx118, BHWX2-2a and Aubusson flours with 37% of moisture content. These traces show that the endothermic peak due to amylose-lipid complexes dissociation was absent for waxy dough samples. In case of waxy dough samples gelatinization peaks appeared at higher temperatures and delimited an apparently larger surface, indicating a higher gelatinization enthalpy variation (ΔH), with respect to that of non-waxy dough. In particular the values of ΔH resulted to be 13.9, 12.4 and 11.2 $J \cdot g^{-1}_{dry}$ respectively for the dough samples from IWWL, American and non-waxy wheats. Nevertheless it has to be mentioned that uncertainties in ΔH measurement were quite high (15%) and do not allow to establish that the ΔH values measured are significantly different.

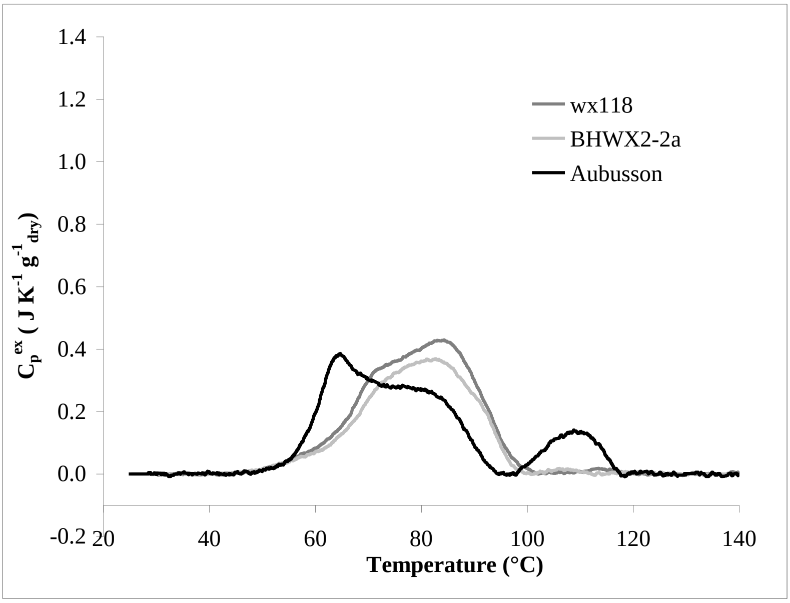

Figure 7. DSC traces of dough from wx118, BHWX2-2a and Aubusson with 37% moisture content.

Figure 8 depicts DSC traces registered for the same dough samples considered under Figure 7 when their moisture content rises to 70%. These traces indicate how the higher amount of water availability lowered gelatinization temperatures.

Results observed by DSC seem to contradict data obtained by RVA which indicated that waxy starches gelatinize at lower temperatures with respect to non-waxy starches. The difference in DSC and RVA results could be due to the different hydration and shear-stress conditions applied in each test. Moreover RVA analysis allows to evaluate not only the mere gelatinization event, as in the DSC analysis, but also the pasting phenomena. Nevertheless it has to be pointed out that 1) the RVA heating rate was 12 °C/min against a heating rate of 2 °C/min considered for the DSC analysis; 2) many small size granules are present in the waxy starch; 3) the percentage of damaged granules is higher in waxy starch compared to non-waxy starch. The difference in the results obtained by DSC and RVA could hence be explained as follows. The high RVA heating rate determined a delay in the time at which non-waxy starch achieves gelatinization conditions due to the larger size of its granules with respect to waxy starch. This delay was further increased by higher heat transmission rate within waxy starch due to the higher percentage of damaged granules that can be more easily hydrated (Kim et al., 2003)

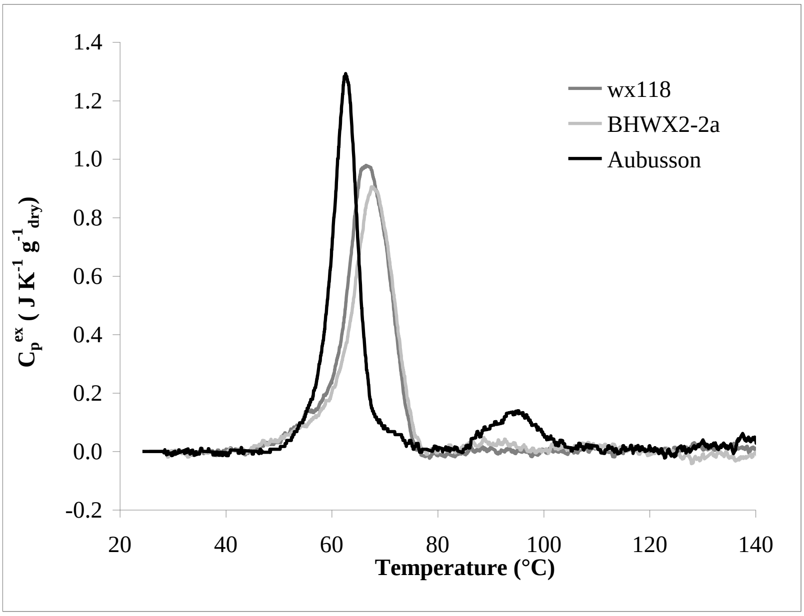

Figure 8. DSC traces of dough from wx118, BHWX2-2a and Aubusson with 70% moisture content.

A description of how water is partitioned within a wheat flour dough can be drawn from Thermogravimetry (TG) investigations. It is indeed well known (Fessas & Schiraldi, 2001) that a wheat flour dough is a heterogeneous system, since it is composed of thermodynamically incompatible polymers (e.g. starch and proteins), which therefore form separate aqueous phases, each of which is richer in a given polymer with respect to the nominal dough composition.

Typical wheat flour dough DTG traces obtained by classical thermogravimetry show two peaks indicating the presence of at least two distinct aqueous phases in the dough. The first peak refers to free evaporation of water (with a fickian diffusion law from the core to the surface of the dough) from the aqueous phase with starch predominance, whereas the second peak refers to water evaporation from the aqueous phase with gluten predominance (Fessas & Schiraldi, 2001).

Figure 9 and Figure 10 show the DTG traces normalized to 100 mg dough samples and obtained for wx118, BHWX2-2a and Aubusson flours respectively with 70% and 40% of moisture content. The two above mentioned peaks resulted not very well differentiated under Figure 9, excepting the dough from wx118 flour for which the second peak was more clearly displaced from the first one and occurred at a higher temperature with respect to the other dough samples. When the dough moisture content was lowered to 40% (see Figure 10), the peak due to water evaporation from the aqueous phase with gluten predominance occurred for higher temperatures and was hence more clearly distinguishable from the “diffusion” peak whose position remained the same observed for a 70% dough moisture content for all dough samples considered. The shift of the second peak towards higher temperatures appears however more pronounced for dough from waxy flour. This indicates that the “force” whereby water is bound to the gluten network increases when dough moisture content decreases and that this “force” was higher for waxy dough (wx118 dough in particular).

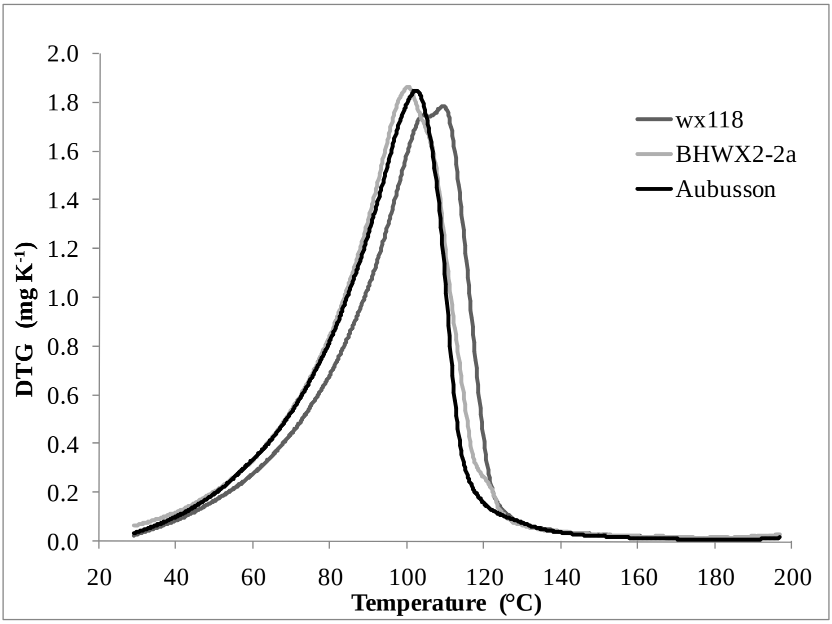

Figure 9 DTG traces of dough from wx118, BHWX2-2a and Aubusson with 70% moisture content.

Figure 11 reports the same DTG traces normalized to 100 mg water content and shows that also in this case the second peak occurred at higher temperatures for wx118 dough. This indicates that water was more tightly bound to the gluten network in waxy wheat, especially in the wx118 sample. In conclusion this analysis shows that proteins were less hydrated in case of dough samples from waxy flours, especially in case of wx118 dough, with respect to dough samples from BHWX2-2a.

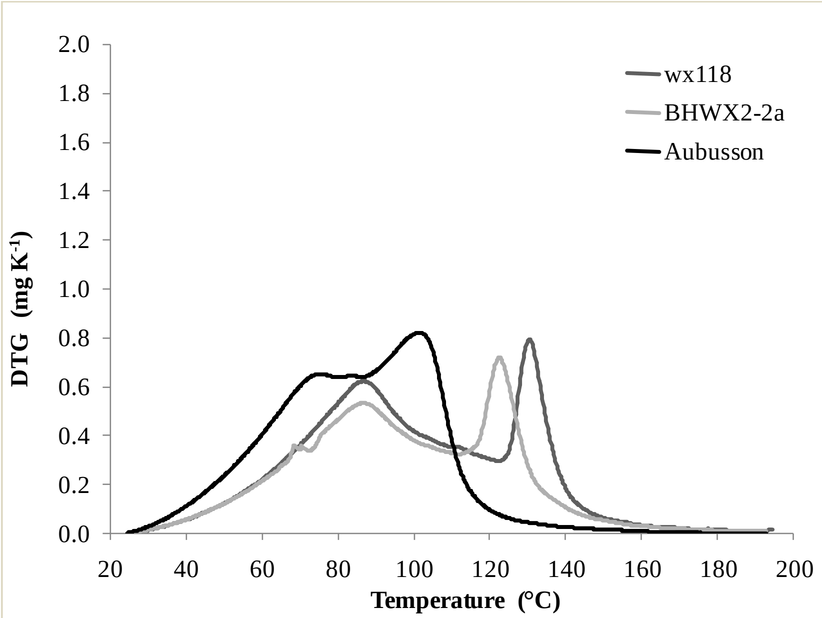

Figure 10. DTG traces of dough from wx118, BHWX2-2a and Aubusson with 37% moisture content.

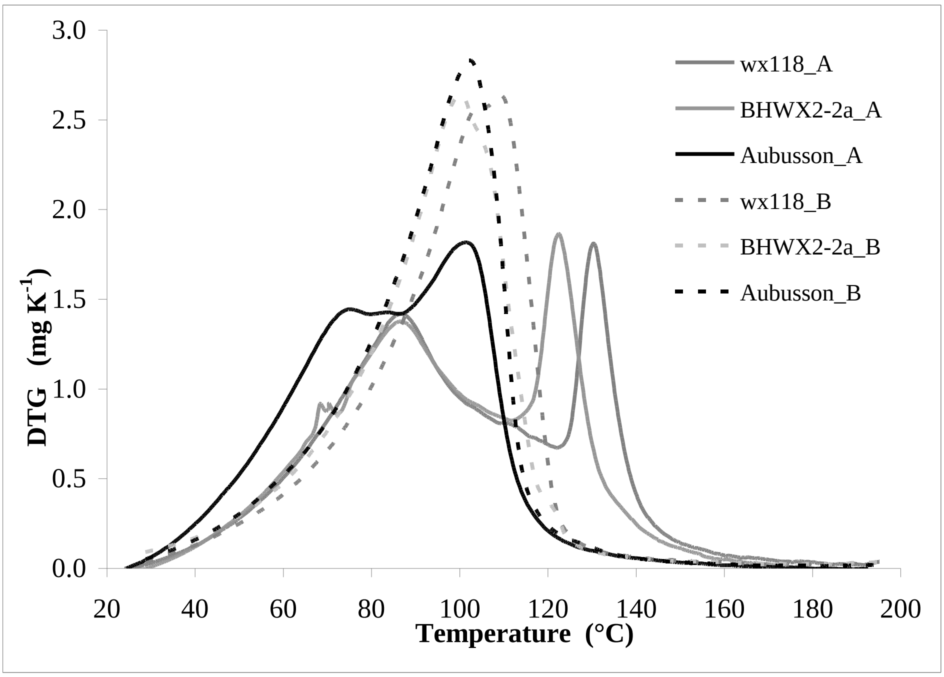

Figure 11. DTG traces normalized to a 100 mg water content of dough from wx118, BHWX2-2a and Aubusson with 37% (A) and 70% (B) moisture content.

3.4 CONCLUSIONS

The low amylose content of all IWWLs considered (markedly below 1.7% d.b) influences the characteristics of starches both in suspensions and in dough systems.

Waxy wheats resulted having starch with more small granules, often with deformities and small cracks with respect to non-waxy wheats.

Regarding the gelatinization temperature, waxy starch gelatinization resulted to take place at higher temperatures (as estimated by DSC analyses) with respect to non-waxy starch. This parameter is strongly affected by the conditions applied in the approach used for its evaluation (e.g. water availability, presence/absence of shear-stress, etc.).

As the retrogradation extent resulted highly reduced in all the IWWL flours, these types of flours could be usefully exploited for baking processes.

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4. PROTEIN CHARACTERIZATION AND RHEOLOGICAL PARAMETERS OF ITALIAN WAXY WHEAT LINES

4.1 STATE OF THE ART

Wheat flour proteins are typically classified into four solubility groups according to a still widely used classification introduced by Osborne (1929): albumins (water-soluble), globulins (salt-soluble), gliadins (alcohol-soluble), glutelins (extracted by dilute acid or alkali). The most represented proteins in wheat flour are the monomeric gliadins and the polymeric glutenins (Sapirstien & Fu, 1998; Singh & Macritchie, 2001; Kuktaite et al., 2004), typically representing 40-50% and 30-40% of flour protein content, respectively, and also defined as prolamins due to their high content in the aminoacids proline and glutamine.

Gliadins are usually classified into α/β-, γ- and ω-gliadins according to their decreasing mobility in Acid polyacrilamide gel electrophoresis (A-PAGE). Glutenins are usually separated through Sodium Dodecyl Sulphate Polyacrilamide Gel Electrophoresis (SDS-PAGE) and classified into high molecular weight (HMW) and low molecular weight (LMW) subunits.

Further classifications of gluten proteins rely on their chemical properties and genetic control. Three groups are defined: sulfur-rich, sulfur-poor, and high molecular wheight (HMW) prolamins. Sulfur-rich prolamins comprises α/β- and γ-gliadins and low molecular wheight (LMW) glutenins. These molecules are characterized by a N-terminal domain, made of short stretch repeats reach in proline and glutamine and a conserved C-terminal domain containing most of the cysteine residues. Gliadin and glutenins components, however, differ in their tertiary structure: gliadins are monomeric units stabilized by intra-molecular disulfide bonds, glutenins are polymeric proteins linked by inter- and intrachain disulfide bonds and other non covalent interactions (hydrogen and hydrophobic bonds) (Shewry et al., 2009). Sulphur-poor prolamins consists almost entirely by ω-gliadins, completely lacking in cysteine and having monomeric structure.

HMW prolamins are made of a central ripetitive domain flanked by non-repetitive domains containing the cysteine residues. Their secondary structure, conferring gluten its peculiar properties, consists of the central part folded in β-sheet, elastic and flexible thanks to the Pro and Gly residues, and the terminal parts rich in α-elix, in which the Cys residues create an elastic network thanks to intermolecular disulfide bonds.

During mixing certain HMW-GS typically undergo repolymerization and depolymerization (Weegles et al., 1996). Formation of dough and, subsequently, its strength are attributed to the polymers formed by intermolecular disulfide bond between HMW-GS and LMW-GS (Wrigley, 1996). Wheat flour proteins interact with each other to form a viscoelastic mass upon hydration (Shewry et al., 2002).

Whereas the role of gluten proteins in the formation of dough has been extensively studied in case of normal wheat (Cornish et al., 2006; Shewry et al., 2009), it is not yet sufficiently clear how protein interactions change and differently affect doughs properties in case of flours from waxy wheats. Moreover most of the studies have so far focused on explaining changes in rheological properties of dough as a consequence of gluten proteins, whereas the role of starch and the effects of starch-protein interactions have not yet been sufficiently investigated.

This study analyses the molecular characteristics of proteins from Italian waxy wheat lines (IWWL) and how these characteristics affect the physico-chemical and rheological properties of related dough.

Waxy wheat proteins have been separated and identified by electrophoresis. Protein molecular characteristics have been then further investigated by using techniques exploiting the different dissociating ability of proteins towards covalent and non-covalent inter-protein bonds in different buffers. Then accessible sulfhydryls (-SH) groups have been considered to understand the structure and reactivity of proteins and to assess the technological properties of related flours. Front-face fluorescence techniques have been instead used to study waxy proteins hydration. Finally waxy dough rheological properties were analysed by subjecting dough to harmonically varying stress or strain in order to assess its visco-elastic properties.

4.2 MATERIALS AND METHODS

Materials

7 Italian waxy wheat lines (IWWL) have been used for the analysis performed, out of which 4 were of hard type (wx70, wx104, wx107, wx128), 2 were of soft type (wx71 and wx118) and 1 of medium type (wx123). Two homozygous waxy wheat WQL6K107-BHWX14-7, PI 612546, hard type (indicated as BHWX14-7 in this study), and WQL6K107-BHWX2-2a, PI 612545, soft type (indicated as BHWX2-2a in this study), kindly provided by C. Morris (Washington State Univ., Pullman, WA) (Morris & Konzak, 2001) were used as control in all analyses together with the non-waxy *cv* Aubusson (ordinary bread making quality, wild type, wt) and a commercial flour of high bread-making quality.

Methods

Analysis of flours

The genotypes were grown in St. Angelo L. (Italy) during the 2009-10 growing season. The chemical proximate composition was determined on flour prepared by milling the grain with a Bona Quadrumat Labormill (Bona, Monza, Italy). Protein content (N x 5.7 dry weight, AACC 39-10, AACC International, 2000) was determined by NIR System Model 6500 (Foss NIR Systems, Laurel, MD). The SDS sedimentation volume (SSV) was determined according to Preston et al. (1982). The specific SDS sedimentation volume (SSSV) was calculated as the ratio between SSV and protein content, whereas flour Gluten Index (AACC 38-12) was estimated by the Glutomatic 2200, the Gluten Index Centrifuge 2015 and Glutork 2020 (Perten Instruments, Stockholm, Sweden). Each test was performed at least two times.

Electrophoretic Characterization

Gliadins were extracted as described by Pogna et al (1990); 30 mg of flour were dissolved in 100 µL of aqueous 70% ethanol and left for 1 h at room temperature. After centrifugation for 5 min at 12,718×*g*, the supernatant was mixed with an equal volume of a solution containing 60%

(w/v) glycerol and 0.05% (w/v) pyronin G, and separated by acid-polyacrylamide gel electrophoresis (A-PAGE) at pH 3.1. For the extraction of the glutenin fraction, the pellet was resuspended in 750 µL of sample buffer (57 mM Tris HCl, pH 6.8, 2% SDS, 8.5% glycerol, 0.01% [w/v] pyronin G, 5% 2-mercaptoethanol) for 1.5 h at room temperature and for 30 min at 80 °C. After centrifugation at 12,718×*g* for 15 min, glutenin subunits were fractionated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS –PAGE) using 12.5% acrylamide gels (Pogna et al., 1989). In addition to all experimental materials described, analyses were also performed on single seeds of each waxy line harvested at Sant'Angelo Lodigiano in 2009 to test genetic uniformity of lines.

Aggregation state and thiol content of flour proteins

The solubility of proteins of the flour samples was determined according to published methods (Rondanini et al., 2000; Berti et al., 2004) by suspending 1 g of sample in 20 ml of 50 mM sodium dihydrogen phosphate buffer, 0.1 M NaCl, pH 7.0, and stirring at 25 °C for 60 min. Where indicated protein solubility in urea was determined by adding 6 M urea, and 10 mM dithiothreitol (DTT) the buffer. The amount of soluble proteins was determined spectrophotometrically according to Bradford (1976), on the supernatant obtained from centrifugation of the extracts at 13,000×*g* for 20 min at 15 °C, using bovine serum albumin as a standard. Results are expressed as mg solubilised proteins/g flour db.

Accessible –SH groups were measured directly on suspensions of flour samples of 250 mg in 5 mL of phosphate buffer (50 mM, pH 6.8) containing 0.2 mM 5,5'-dithiobis-(2-nitrobenzoate) (DTNB, Ellman 1959) and 8 M urea where indicated following procedures detailed in Berti et al., (2004). After 15 min of incubation at room temperature, insoluble materials were removed by centrifugation for 15 min at 13,000 ×*g*, the absorbance at 412 nm of the clear supernatant was read against a DTNB blank. Results are expressed as µmol of accessible SH-groups/g of db.

All spectrophotometric analyses were performed by using a double beam spectrophotemeter (Perkin Elmer Lambda 2A, Waltham, Massachusetts, USA) connected to a personal computer. Data were elaborated by the software Perkin Elmer UV WinLab version 2.80.03.

Front-face fluorescence

Solvation of proteins in the different systems under investigation was monitored by taking fluorescence emission spectra of the protein tryptophan and of protein-bound 1,8-anilino-naphthalene-sulfonate (ANS) at increasing water content. Front-face fluorescence was measured at room temperature in a LS 50 B luminescence spectrometer (Perkin-Elmer, Waltham, Massachusetts, USA) according to Bonomi et al., (2004). The standard front-face fluorescence cell provided by the Perkin-Elemer was loaded with about 100 mg of each sample. Tryptophan fluorescence was monitored by taking emission spectra from 300 to 420 nm, with excitation at 280 nm, with excitation and emission slits set at 2.5 nm, and a scan speed of 50 nm/min. Emission spectra of ANS were taken from 405 to 520 nm, with excitation at 390 nm. Emission and excitation bandwidths were set at 5 nm. Solvation studies were performed by adding to individual flour samples enough water to reach a final water content in the 30-50% range. In case of samples prepared for the measurement of ANS emission spectra, part of the water was replaced with appropriate increasing volumes of 0.5 mM ANS. The added liquid was dispersed by careful manual mixing of the wet flour with a glass rod for 3 min.

Rheological properties

Viscoelastic properties of dough prepared from waxy and non-waxy flours were studied by dynamic oscillatory measurements performed on a Physica MCR100 Rheometer (Physica Messtechnic GmbH, Ostfildern, Germany).

The dough was obtained by mixing (1 minute) in the Glutomatic 2200 (Perten Instruments, Stockholm, Sweden) 10g of flour and amounts of water in the same proportions considered in the farinograph analysis. A parallel plate geometry (25 mm diameter, 1 mm gap) was used, with corrugated plates to prevent dough slippage. The temperature was regulated at 25 °C by a circulating bath and Peltier-based temperature control. After loading, the excess dough was trimmed and a thin layer of paraffin oil was applied to the edge of the exposed sample to prevent moisture loss during measurements. The dough was allowed to rest for 30 min in order to equilibrate stresses, before starting the test. The following tests were performed on each sample: strain sweep tests, in the range of 0.01–200%, at 1 Hz frequency; frequency sweep tests, in the range of 0.1–10 Hz, at 0.03% strain. Each test was performed at least three times. Data were analyzed using US200/32 v.2.50 rheometer software (Physica Messtechnic GmbH, Ostfildern, Germany).

Statistical analyses

The analysis of variance was performed by using the Statgraphics Plus 4.0 package. Least Significant Difference (l.s.d. p=0.05) was used to compare the mean values of the parameters considered.

4.3 RESULTS AND DISCUSSION

The protein content and the values of some qualitative indices typically used to assess the technological performances of flours are reported in Table 1.

The IWWL flour protein content resulted on average quite high, as also found by Van Hung et al. (2006), and ranged from 12.0% to 15.4%. The SSV values of IWWL flours resulted mostly comparable to those of non-waxy flours except for the wx71 flour which gave markedly higher SSV values. The wx71 flour gave the highest values also for SSSV with respect to other IWWL flours and to controls. All IWWL gluten index values resulted significantly higher than values reported for BHWX14-7 flour and were comparable or slightly lower with respect to those given for non-waxy flour, excepting wx104 flour, that gave much lower gluten index values.

Table 1. Characterization of the flours analysed by protein content and some qualitative indexes.

Lines		Wholemeal protein content (% d.b.)	Flour protein content (% d.b.)	SSV (mL)	SSSV (mL)	Gluten index (%)
wx70	wx	12.4±0.1 b	12.0 ±0.1 c	78±4 b	6.5±0.3 def	68±2 c
wx71	wx	13.3±0.2 c	11.6 ±0.1 b	94±1 d	8.0±0.1 h	76±4 def
wx104	wx	16.5±0.3 f	15.4 ±0.1 i	85±1 c	5.5±0.1 a	51±1 b
wx107	wx	14.8±0.1 e	14.0 ±0.1 h	93±1 d	6.6±0.1 f	82±2 fg
wx118	wx	14.8±0.1 e	12.9 ±0.1 e	81±1 b	6.3±0.1 cd	70±2 cd
wx123	wx	14.7±0.1 e	13.2 ±0.1 f	88±2 c	6.6±0.2 ef	79±5 ef
wx128	wx	14.1±0.1 d	12.9 ±0.1 e	81±1 b	6.3±0.1 cde	79±1 ef
BHWX14-7	wx	14.5±0.2 e	13.3 ±0.1 f	81 ±1 b	6.1±0.1 bc	44±2 a
BHWX2-2a	wx	14.1±0.3 d	12.2±0.1 d	66 ±1 a	5.4±0.1 a	-
Aubusson cv	wt	12.2±0.2 a	10.8 ±0.1 a	79 ±2 b	7.3±0.2 g	74±6 cde
Comm. Flour	wt	-	13.7 ±0.1 g	80 ±1 b	5.9±0.1 b	89±2 g

Values followed by same letter in the same column are not significantly different (P<0.05).

Results of the analysis of HMW-GS composition of IWWL are shown in Figure 1. The most common subunit composition was represented by allele c (null) at HMW-GS Glu-A1 locus, allele b (subunits 7+8) at Glu-B1, and allele a (subunits 2+12) at Glu-D1. This subunit combination corresponds to that observed for the BHWX14-7 line used during the crosses whereby the IWWL considered in this study have been generated. It is worth noting that the subunits 2+12* were observed in the lines wx123, wx128 and wx104 and that the subunit 12* is quite rare and has a slightly higher electrophoretic mobility with respect to subunit 12. In general the subunit composition observed here appears with high frequency in Italy and not in the USA (Shan et al., 2007) and is associated to medium bread making quality. Indeed, the average score assigned to this composition is 8 according to the scoring system introduced by Pogna et al. 1988, which associates an overall score to glutenin subunits depending on their bread making qualities (table 2). This scoring system is based on the pattern of HMW glutenin subunits separated by SDS-electrophoresis. The full score for a specific genotype can be calculated by adding the individual scores of the alleles at each of the three Glu-1 loci, giving a total ranging from 4 to 17.

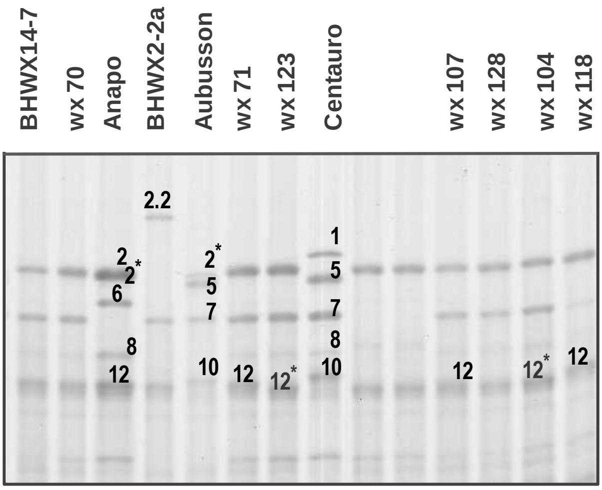

Figure 1 Separation of glutenin subunits (HMW-GS) of wheat lines considered in this study by one-dimensional SDS-PAGE. HMW-GS composition of cultivars Centauro and Anapo were used to deduce the composition of the lines analysed.

Table 2. Total score assigned to High Molecular Weight Glutenin Subunits (HMW-GS) of the wheat samples (according to Pogna et al.,1988).

Wheat	HMW-GS composition	Quality score (range: 4-17)
Italian waxy wheat lines	**N, 7+8, 2+12 or 2+12***	**8**
BHWX14-7	**N, 7+8, 2+12**	**8**
BHWX2-2a	**N, 7, 2.2+12**	**6**
Aubusson cv	**2*, 7, 5+10**	**13**

Gliadins may represent approximately 50% of the gluten proteins and several studies have concluded that gliadins affect extensibility and viscosity of gluten and dough (e.g. Mills et al., 1990). Results of the electrophoretic analysis by A-PAGE of gliadins extracted from the IWWL are reported in Figure 2. The different electrophoretic mobilities observed allowed separating the different gliadin types. All IWWLs considered have a samilar gliadin composition, except for small differences in the region of the β- and γ-gliadins. In particular all waxy lines have the γ-component 43.5 which seems to be associated to a good protein quality (Bushuk & Zillman, 1978). None of the IWWLs inherited the chromosome translocation 1BL/1RS which was instead observed in the cultivar Barra used in the crosses whereby the IWWLs have been generated (see chapter 2).

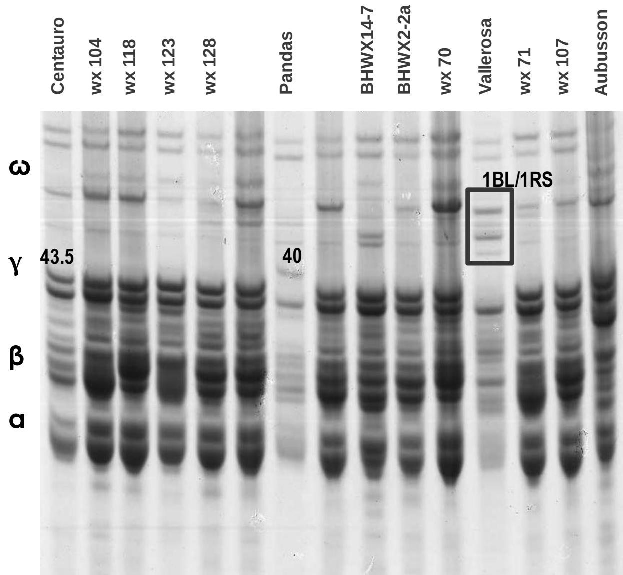

Figure 2. Separation of the gliadin components of the wheat lines considered in this study by one-dimensional A-PAGE. The composition of cultivars Centauro, Pandas and Vallerosa were used to deduce the composition of the lines analysed.

The nature of proteins and the relevance of the diverse inter-protein interactions that contribute to the features of dough was further investigated.

The solubility of proteins in waxy flour was also evaluated by extraction in buffers with different dissociating ability towards covalent and non-covalent inter-protein bonds. This differential-solubility approach allows a course correlation between the physico-chemical properties of cereal proteins, as inferred from their aggregation state and their behavior during food processing (Rondanini et al., 2000; Alamprese et al., 2005).
Solubility of native flour proteins in plain phosphate buffer offers hints about modifications affecting water soluble protein components (albumins and globulins). Solubility in urea, in presence and in the absence of disulfide-reducing compounds (DTT), along with the effects of the disulfide-reducing agent, provides information about covalent and non-covalent interactions between intrinsically water-insoluble proteins, such as gliadins and glutelins in flour (Alamprese et al., 2005).
The solubility data of some IWWLs considered in this study are presented in Figure 3. This figure shows that in case of waxy wheat proteins the differential-solubility (i.e. the difference between solubility in urea in presence and in absence of DTT) varies among the different waxy lines (both Italian and American ones) and that non waxy protein differential-solubility falls generally within a same variability range.
It may be interesting to notice that proteins from wx104 flour have the highest relative solubility in urea solution, indicating the presence of few inter-protein disulfide bonds in protein

aggregates, whereas proteins from wx128 and BHWX2-2a flour have the lowest relative solubility in urea solution indicating the presence of several inter-protein disulfide bonds in protein aggregates. This has been confirmed also by electrophoretic analyses (data are not showed) of proteins extracted in buffers (urea, urea+DTT).

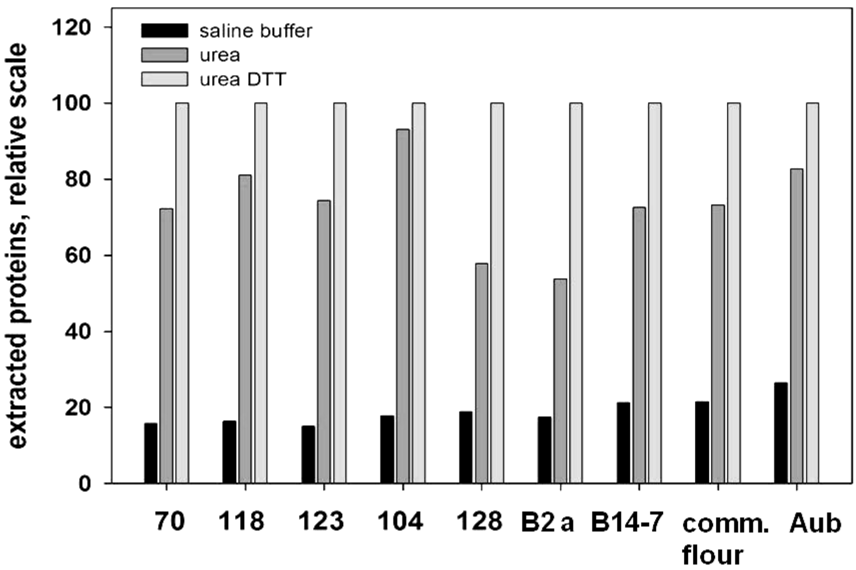

Figure 3. Solubility of waxy wheat flour proteins in different buffers (commercial flour and Aubusson cv were used as reference)

Sulfhydryls (–SH) and disulfides (–S–S–) groups play an important role in the structure and reactivity of food proteins, and, consequently, in the technological properties of flours (Pomeranz,1994; Schofield, 1994). The accessible sulfhydryls (-SH) groups were measured directly on suspension of flour in phosphate buffer, so that only free cysteines could be analysed. In the case of the samples considered here, it was not possible to evaluate the total thiols content in presence of urea, as reported in Berti et al., (2004), because the urea increased sample viscosity and did not allow to perform this evaluation.

The arrangement of thiols in the waxy flours was studied by assessing the amount of –SH groups reactive towards the bulky thiol reagent DTNB after 15 min of reaction, as reported in Figure 4. The highest accessibility of sulfhydryls groups is observed in BHWX14-7 and BHWX2-2a flours, whereas accessibility of sulfhydryls groups seems to be variable for the IWWL and seems comparable to that of non waxy flour. In case of wx118 the values of –SH groups accessibility resulted very low. Nevertheless all doughs from IWWLs, wx118 included, produce a bread of better quality with respect to American waxy dough (see chapter 5).

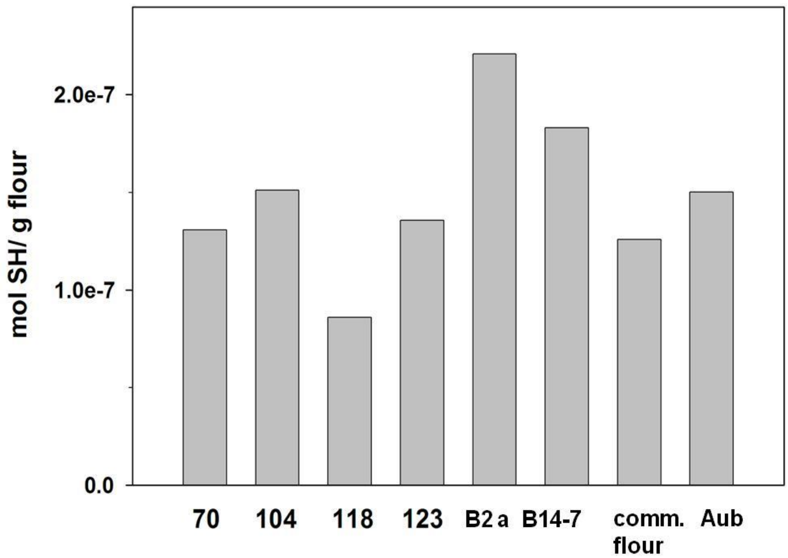

Figure 4. Accessibility of thiols in saline buffer of waxy wheat flour (commercial flour and Aubusson cv were used as reference)

Solid-state fluorescence, also known as front-face fluorescence, has been extensively used in view of its capacity to monitor subtle changes in chemical or physical environment of reporter fluorescent molecules, that can be a part of the protein structure (as is in case of the tryptophan side chain) or can be contained in external probes (Bonomi et al., 2004). By this approach it is possible to evaluate the ability of the proteins to change their conformation as a function of their hydration, also in case of solid food systems as dough. In this case insoluble gliadins and glutelins play a fundamental role in assessing the properties of the starting material and of the products obtained by processing, because protein characterization by other approaches (e.g. methods implying extraction/separation steps) has intrinsic limitations for the preservation of the native protein structure. The water added to flour allows to solvate some of the proteins in the system and to expose those regions of insoluble proteins that are involved in the formation of an interprotein network through the controlled application of mechanical deformation (Bonomi et al., 2004). Regions containing hydrophobic residues have a forefront role in these events.

The changes in tryptophan front-face fluorescence spectra with increasing amounts of added water are shown in Figure 5, only for wx104 flour.

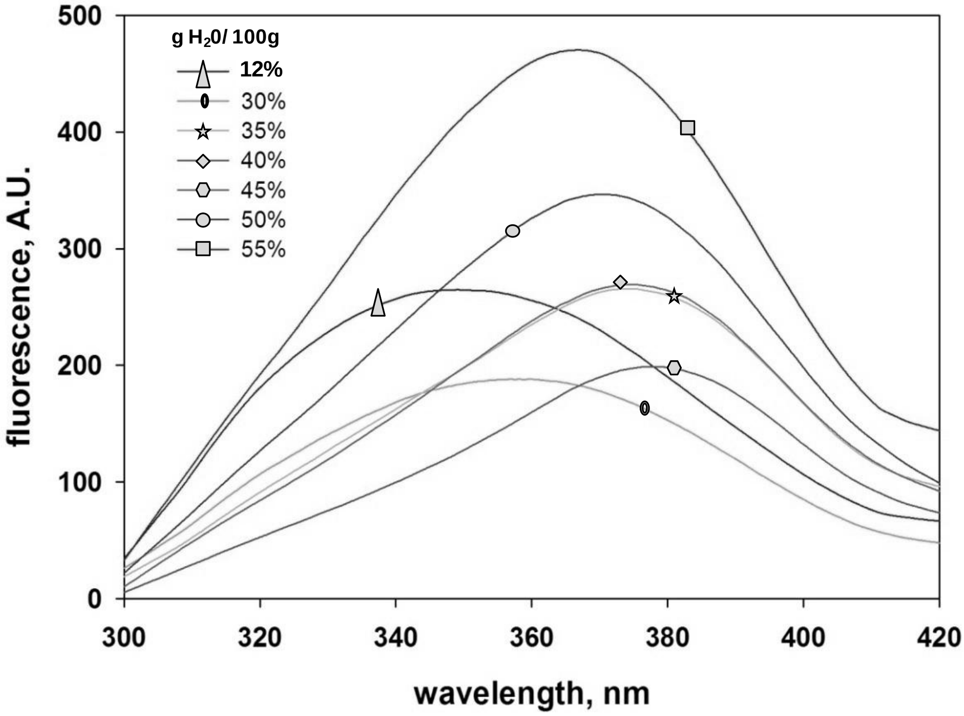

Figure 5. Tryptofhan fluorescence spectra of wx104 flour with increasing amounts of water .

Increased protein hydration allows the reorganization of the protein structure. This conformational change can be monitored as increased solvent exposure leads to a shift of the tryptophan fluorescence maximum towards longer wavelengths. The position of the intensity maximum in tryptophan fluorescence spectra is therefore related to the chemical environment of the tryptophan side chain. Exposure to solvent water gives a remarkable red shift of the emission maximum with respect to a residue buried in the protein interior. The fluorescence intensity is influenced by the surrounding medium and by presence of nearby residues that may act as "quenchers" of the emission phenomenon and lower the quantum yield (Bonomi et al, 2004). Figure 6 reports the tryptophan emission maximum as a function of the amount of added water for some of the waxy flours analysed.

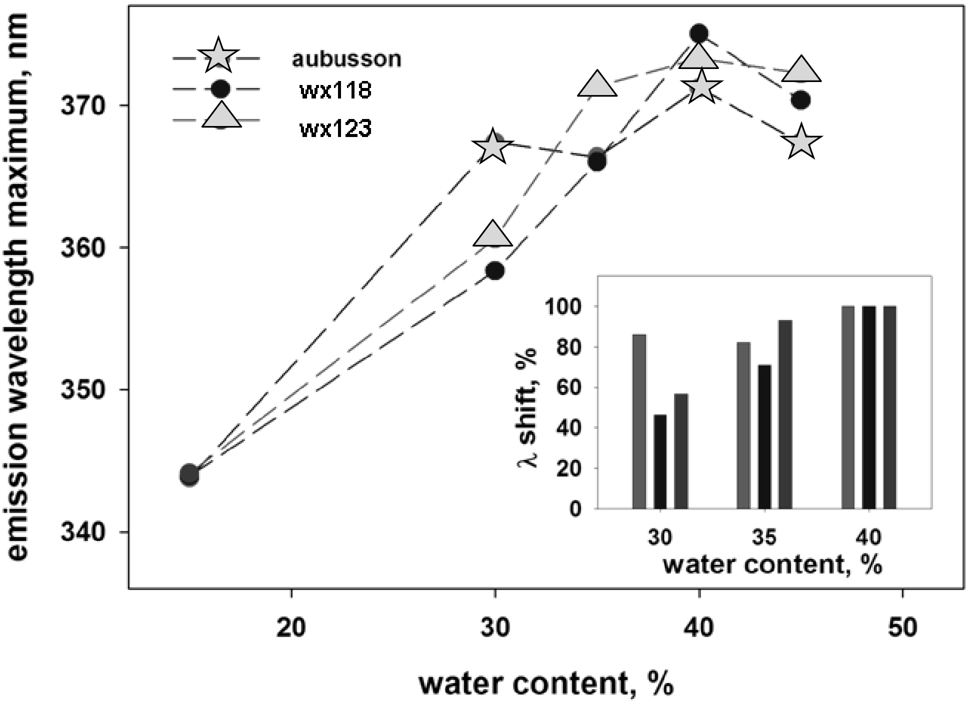

Figure 6. Tryptofhan fluorescence spectra of waxy flour after addition of various amounts of water .

It should be noted that the proteins of non-waxy samples are completely hydrated at a 30% water content (the emission maximum does not increase for higher water contents as shown by the bar full height in the box under Figure 6), whereas full hydration is reached at about 35% of water content for waxy wheat lines. Considering that the difference in the protein content of waxy and non-waxy flour should not cause this different behavior, this discrepancy can be explained by assuming that other flour molecular components (e.g. the waxy starch itself) retain the water needed for protein hydration. Furthermore, changes in protein structure in the waxy samples seems to show a sigmoidal dependence on the amount of added water (contrary to the "saturation" behavior of the non-waxy specimen). This suggests that other macromolecules may compete for available water at low water contents. Of course, starch is a prime candidate for this effect.

It is indeed widely accepted that starch and protein compete for available water during the initial stage of dough formation. Waxy starches contain mostly amylopectin and swell faster and more as compared to normal starch granules. Consequently, dough made from waxy wheat flours displays higher water absorption as compared to normal wheat flours (Guan et al., 2009). This hypothesis seems to be confirmed also by the results of the DTG analysis illustrated under chapter 3 indicating that waxy starch retains more water than non-waxy starch. In this respect it is finally worth mentioning that an adequate level of protein hydration is generally considered as the necessary prerequisite to produce a dough with good technological properties.

To study changes in the mutual arrangement of hydrophobic residues other than tryptophan upon solvation of wheat flour, the fluorescence emission of ANS was monitored at fixed ANS concentration and increasing water contents (Bonomi et al., 2004). As shown in figure 7, a progressive increase of the water content resulted in progressive increase in the fluorescence intensity of ANS in case of dough from non-waxy flour. In case of dough from waxy flours the fluorescence intensity at 30% of moisture content is instead significantly higher with respect to non-waxy flour, but it decreases markedly up to 35% of moisture content and then increases again at higher moisture. The higher initial fluorescence intensity of doughs from waxy flours might be caused by a partial interaction between protein hydrophobic regions and partially solvated regions of amylopectin. These interactions may decrease when amylopectin is completely solvated at higher water content in the system. However, further investigations are needed to explain this difference between ANS-related fluorescence in dough from waxy and non-waxy flours.

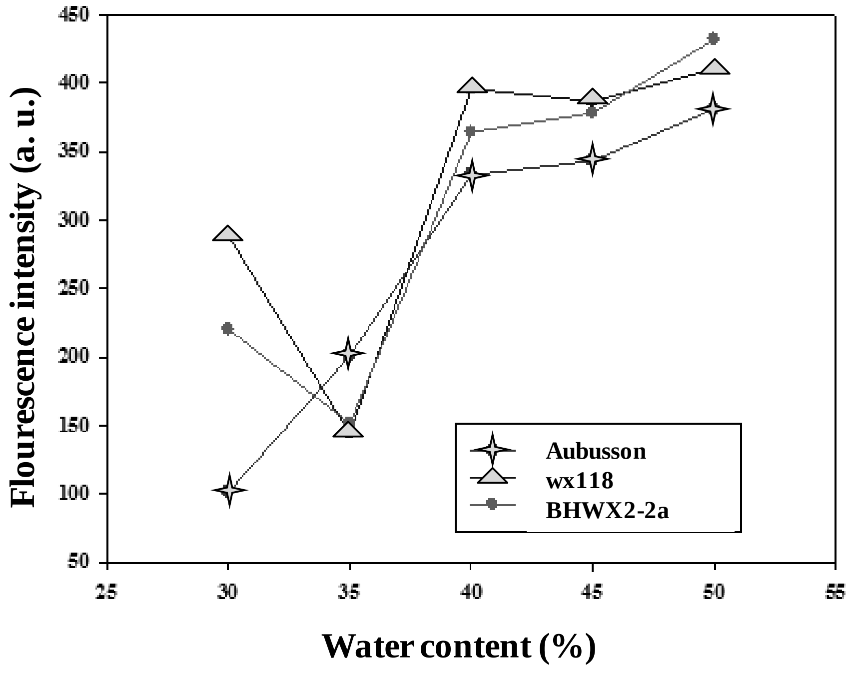

Figura 7. Protein solvation in doughs from wx118, BHWX2-2a and Aubusson flours as estimated by ANS binding evaluated through front-face fluorescence.

Dough rheological properties

Gluten is the structure-forming complex in wheat, responsible for the viscoelastic properties needed to produce good quality baked products. Interactions of gliadins and glutenins through covalent and non-covalent bonds to form gluten complexes result in viscoelastic dough that has the ability to withstand stresses applied during mixing and to retain gas during fermentation and baking, producing a light baked product (Lindsay & Skerritt, 1999). During oscillatory measurements, samples are subjected to harmonically varying stress or strain and results are usually sensitive to chemical composition and macromolecular physical structure. Dynamic tests are used to calculate the frequency-dependent viscoelastic moduli G′ (storage modulus or elastic modulus) and G″ (loss modulus or viscous modulus), which are respectively the real and imaginary parts of the complex shear modulus $|G^*|= |G' + iG''|$. The loss tangent or damping factor (DF) is defined as: $\tan \delta = G''/G'$ (Mariotti et al., 2009).

The evaluation of the region of linear viscoelasticity (LVE) of a sample is a fundamental step. When materials are tested in their linear range, their characteristics do not depend on the magnitude of the stress, the magnitude of the deforming strain, or the rate of application of the strain: if linear, an applied stress will produce a proportional strain response (Steffe, 1996). The LVE of waxy dough samples was identified up to 0.03% strain.

In order to have a rheological characterization of dough, frequency sweeps have been performed to show how the viscous and elastic behavior of the dough change with the rate of application of strain or stress, while the amplitude of the signal is held constant (Steffe,1996). The frequency sweep curves of some IWWL and non waxy dough samples are shown in figure 8.

G′ was strictly frequency-dependent and increased with increasing frequency, while for G″ this effect was evident only at higher frequencies. As G′ and G″ values were very different, their ratio (tan δ) was very low.

For all samples in the range of frequencies considered, G′ was greater than G″ suggesting a solid elastic-like behavior of both waxy and non waxy dough. The dough from wx71 flour had the highest values of G′ among all waxy dough, showing a behavior very similar to that of dough from non-waxy wheat. It's worth noting that this sample was characterized by a high Brabender stability and originated bread characterized by a high specific volume, as previously reported (Chapter 5). Lower and decreasing values were registered respectively for dough from wx123, wx70 and BHWX2-2a flour, with wx118 dough resulting the least elastic together with BHWX14-7. The same ranking was basically observed also in relation to values registered for G″. The dough from wx71 flour gave the lowest value for tan δ among all waxy dough samples indicating a more elastic material.

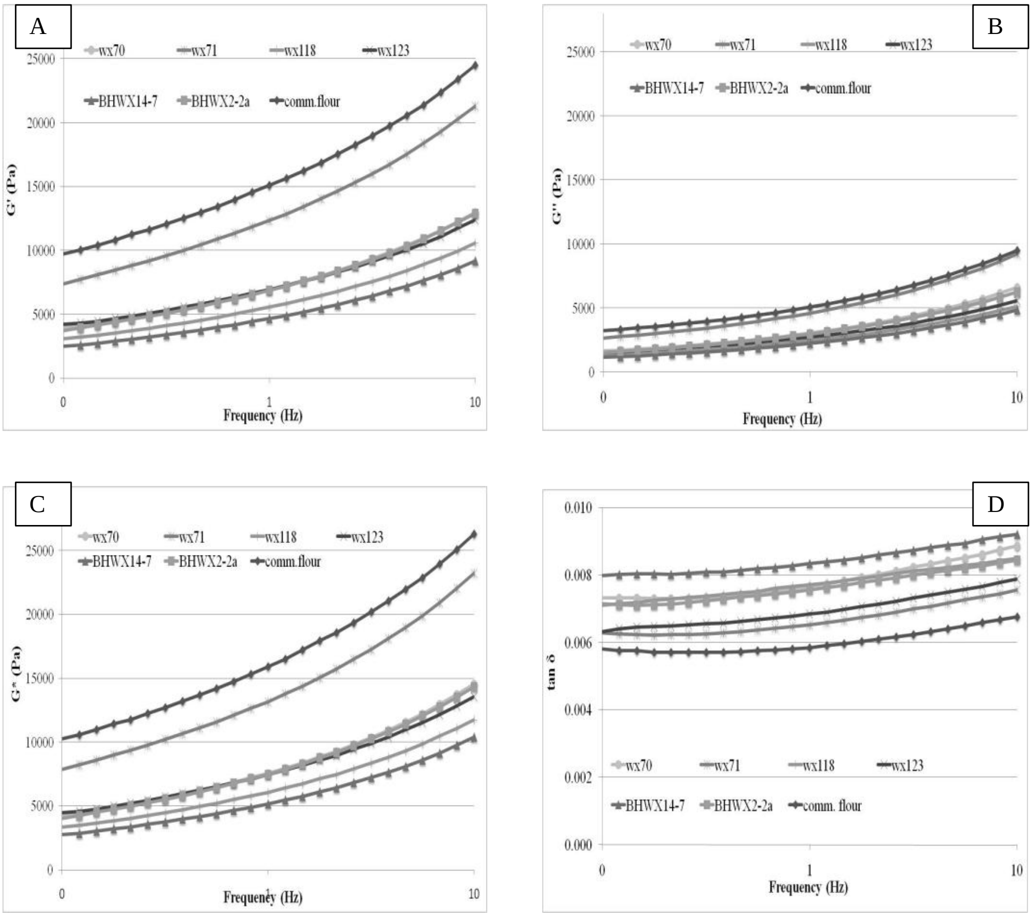

Figure 8. Frequency sweep curves (0.03% strain, 25°C) of waxy dough and commercial dough (curves are the mean of at least two replicates). A: Storage modulus, G′ (Pa); B: Loss modulus, G″ (Pa); C: Complex modulus, G* (Pa); D: Damping factor, tan δ.

The results of the application of an exponential equation (power law equation: $y=ax^b$) that fits very well (r >0.95) all the frequency sweep curves are reported in Table 3. The used variables were: frequency (x) and G′ or G″ (y). The parameter "a" is a consistency index and the exponent "b" is related to the strength and the nature of the dough (Mariotti et al., 2009). The dough from non-waxy commercial samples and wx71 had the highest G′ consistency indices, whereas the lowest "a" values occurred for BHWX14-7 dough. With regard to "b" values, the higher the value of the exponent, the higher the dependence of the structure on strain: the less sensitive were the dough from non-waxy, wx71 and wx123 wheats. Dough from wx118 and American waxy wheat samples had higher "b" values, which could be associated with a lower elasticity in comparison to the other dough considered.

Table 3. Application of the power law equation to the frequency sweep test of G′ (storage modulus) and G″ (loss modulus) for the dough samples analysed.

Dough	G'		G"	
	a	b	a	b
wx70	6996 b ± 38	0.258 c ± 0.002	3130 b ± 57	0.302 c ± 0.004
wx71	12425 c ± 1055	0.226 b ± 0.014	4722 c ± 117	0.270 b ± 0.016
wx118	5604 ab ± 269	0.266 cd ± 0.004	2482 ab ± 82	0.306 c ± 0.006
wx123	7004 b ± 544	0.235 b ± 0.008	2788 ab ± 279	0.282 b ± 0.003
BHWX14-7	4814 a ± 330	0.278 d ± 0.008	2297 a ± 113	0.310 c ± 0.008
BHWX2-2a	6899 b ± 730	0.264 cd ± 0.001	3012 b ± 294	0.305 c ± 0.002
Comm. Flour	15186 d ± 1515	0.197 a ± 0.004	5227 c ± 602	0.234 a ± 0.003

Values followed by same letter in the same column are not significantly different (P<0.05).

4.4 CONCLUSIONS

The protein content of IWWL flours considered in this study resulted - on average - quite high, ranging from 12.0% to 15.4%, whereas the values of IWWL flour qualitative indexes (SSV and gluten index) resulted comparable to those of non-waxy flours and mostly higher than those registered for the American waxy flours. Despite the high protein content of IWWL, HMW-GS composition indicated medium bread making quality. From the results of the electrophoretic analysis, all IWWLs presented a similar gliadin composition, except for small differences in the region of the β- and γ-gliadins. Differential-solubility data showed no marked differences between waxy and non-waxy proteins and also accessibility of sulfhydryl groups seemed to be comparable between IWWL and non-waxy wheat proteins.

Front-face fluorescence analyses allowed instead to highlight an interesting difference between IWWL and non-waxy wheat proteins, showing that dough from IWWL flours needed more water to completely hydrate their proteins, probably as a consequence of the particular composition of waxy wheat starch. The particular waxy wheat starch composition probably affected the interactions between protein hydrophobic regions and partially solvated regions of amylopectin. Finally, rheological analyses indicated that for all samples the elastic modulus prevailed over the viscous modulus at all frequencies considered. Moreover, non-waxy flour dough resulted characterized by a much more solid elastic-like behavior with respect to waxy flour dough. Among waxy samples, dough from IWWL flour, in particular wx71, presented higher consistency and elasticity indices with respect to American waxy dough.

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5. BREAD MAKING CHARACTERISTICS AND STALING KINETICS OF ITALIAN WAXY WHEAT LINES

5.1 STATE OF THE ART

Waxy (amylose-free) wheat (*Triticum aestivum* L.) is considered to have interesting functional properties for bread-making, resulting in formation of a new texture of breads with softer breadcrumbs and with retarded staling susceptibility. These aspects are relevant for bakers and supermarkets which may increase their economic returns by and extended shelf life of backed goods. Moreover quite recent studies have focused on the application of waxy wheat for making whole-grain breads because of the high amounts of dietary fibre, resistant starch, vitamins, minerals and micro-constituents located in the bran and germ of waxy wheat grains (Hung et al. 2007 a, Hung et al. 2007 b).

Bread staling is usually characterized by toughening of the crust, firming of the crumb, loss of moisture and flavor resulting in a loss of product freshness (Hoseney 1994). Bread staling depends primarily on a change in the structure of starch from an amorphous structure to a partially crystalline state (Thomas and Atwell 1997).

Bread staling retardation is generally mainly attributed to a reduction of starch amylose content (Lee et al. 2001; Morita et al. 2002b). For this reason several researches have considered the effects associated with the use of waxy flour in bread-making. Studies on the staling of breads using waxy starch to change amylose/amylopectin ratios of flour were indeed widely performed (Bhattacharya et al. 2002; Ghiasi et al. 1984; Hayakawa et al. 2004; Lee et al. 2001; Morita et al, 2002b). These studies reported that the incorporation of waxy wheat starch resulted in a retardation of staling due to higher moisture retention in breadcrumbs during storage and to a slower kinetics of staling.

Breadmaking properties of flours made only by waxy wheats were analysed in particular by Morita et al., (2002 a) and Hung et al. (2007a)

The present study focuses on the Italian context and considers three out of the best waxy wheat lines obtained by a breeding project aiming at producing waxy wheat *genotypes* that could be proposed for cultivation in Italy. The properties of dough and the quality of bread obtained by using these lines were compared with that of two American waxy wheats. Breadmaking quality has been analysed also in relation to bread staling.

5.2 MATERIALS AND METHODS

Materials

Three Italian waxy wheat lines (IWWL) have been used: wx70, wx118 and wx123 respectively hard, soft and medium type. Two homozygous waxy wheat WQL6K107-BHWX14-7, PI 612546, hard type (indicated as BHWX14-7 in this study), and WQL6K107-BHWX2-2a, PI 612545, soft type (indicated shortly as BHWX2-2a in this study), kindly provided by C. Morris

(Washington State Univ., Pullman, WA) (Morris & Konzak, 2001) were used as control in all technological analyses together with the non waxy cultivar Aubusson (wild type, wt) and a commercial flour of high bread-making quality. The genotypes were grown in St. Angelo L. (Italy) during the 2009-10 growing season.

Methods

Flour characterization

Flour was prepared by milling the grain with a Bona Quadrumat Labormill (Bona, Monza, Italy). Flour samples were characterized by several chemical and physical indices. Protein content (N x 5.7 dry weight, AACC 39-10, AACC International, 2000) was determined by the NIR System Model 6500 (Foss NIR Systems, Laurel, MD). Amylose and total starch content were determined enzymatically using the kit Megazyme International and the "Total Starch assay Kit (AACC76-13, 2001) respectively (Megazyme International Ireland Ltd., Bray Business park, Bray, Co. Wicklow, Ireland). Fat content was determined after acid hydrolysis (AACC 30-10, 2001). Ash content was determined according to official standard method AACC 08-12 (2001). The SDS sedimentation volume (SSV) was determined according to Preston et al. (1982). The Gluten Index (AACC 38-12) was estimated by the Glutomatic 2200, the Gluten Index Centrifuge 2015 and Glutork 2020 (Perten Instruments, Stockholm, Sweden). The rheological evaluation was completed by the Brabender Farinograph test (Brabender OHG, Duisburg, Germany) using a 50g mixer according to the ICC 115-D (ICC 1992).

Leavening test

The leavening test was performed by using 6 small dough samples (about 10.0 g) obtained according to the bread-making procedure described in the following section. These small samples were confined to Petri dishes for 4 hours at 30°C and at a relative humidity of 80%. Dough samples surfaces were monitored and evaluated every 30 minutes by a flatbed scanner (Epson Perfection 3170 Photo, Saiko-Epson Corporation, Japan) and digital images were processed by image analysis techniques (Riva et al., 2004) using the software Image-Pro PlusTM (release 4.5.1.29, Media Cybernetics Inc., Silver Spring, MD, USA). Images were scanned full scale in 256 gray levels at 150 dots per inch (dpi). Surface values in cm^2 were calculated by considering that 150 dpi correspond to 59.10 pixel/cm. A Best Fit Equalization has been used to maximize the difference between dough and image background. Parameters considered for this test were the total number of cells, the number of cells smaller than 0.5 mm^2, the number of cells with a surface between 0.5 and 2.5 mm^2 and the number of cells larger than 2.5 mm^2.

Bread making procedures

Bread loaves from 25 g of flour were produced according to Method 10-10B (AACC International, 2000) with some modifications (e.g. in the absence of milk and potassium bromated) (Borghi et al., 1996) introduced to make the method more similar to the one traditionally used in Italy. The baking formula (flour basis) was flour 50.0g (14%db), sugar 2.0 g, salt 1.0 g, shortening 1.5 g, yeast 2.0 g and 2 ppm of ascorbic acid. The water added to form optimum dough was calculated from the flour absorption index during a Farinograph test performed to obtain an optimal consistence of 500 BU. All ingredients were mixed for 3 min. The dough was divided into two pieces (about 45 g/piece) and then fermented in cabinet at 30°C at a 80% relative humidity for 150 min. The punching was done two times during the

fermentation (after 50 min and after 75 min). All dough samples were placed in apposite baking pans (4.2 x 7.0 cm in top, 3.1 x 6.0 cm in bottom, and 4.0 cm in depth) and fermented for 70 min before baking. Dough pieces were baked at 220°C for 20 min. Bread loaves were allowed to cool for a minimum of 60 min before further test. Baking losses were estimated as the percentage variation of dough weight before and after baking.
A commercial flour of high bread-making quality was used as control for all technological analyses.

Bread quality evaluation

Color analysis
A color meter (CR 210, Minolta Co., Osaka, Japan) was used to measure the lightness and saturation of the color intensity value of bread using the CIE-LAB uniform color space procedure. CIE-Lab-System color value L*, a* and b* as measures of lightness, redness-greenness, and yellowness-blueness, respectively, were recorded for each sample and compared with a white standard. Each measurement was replicated five times and the average value was used.

Moisture content and water activity
The moisture of bread crumb and of the bread were determined according to the official standard methods AACC 44-15 (2001). The water activity of bread crumb was determined by LabMaster-aw (Novasina AG, Lachen, Switzerland) at 25°C and has been measured after 5 min of stability in the detected water activity. Each measurement was replicated five times and the average value was used.

Bread volume and slice cell area
The volume of each loaf was measured by rapeseed displacement. For the staling study, the loaves were placed in perforated plastic bags (15x20 cm), which were subsequently sealed and stored at 22°C.
On day 1, day 3 and day 7 after baking, two loaves of each formulation were sliced into 2.5 cm thick slices. Characteristics of bread crumb were determined on slices from the middle by using a scanner (Epson Perfection 3170 Photo, Saiko-Epson Corporation, Japan) and the image analysis instrument used for the leaving test. Slice image and data on number of gas cells and cells' area were obtained. Parameters considered were the total number of cells, the number of cells smaller than 0.5 mm^2, the number of cells with a surface between 0.5 and 3.0 mm^2 and the number of cells larger than 3.0 mm^2. Firmness, water activity and moisture of every loaf crumb were evaluated after breadcrumb images scanning.

Texture analysis
Firmness was measured by a modified AACC 74-09 method (AACC International, 2000). Bread slices were tested using a Texture Analyzer TA-HD- plus (Stable Micro System Ltd, UK) with a 36 mm cylindrical probe. The firmness of the bread samples was evaluated as the force (N) required to compress the bread slices (25 mm thick) with a cylindrical probe up to 30% of deformation. Firmness values reported were the average of four measurements.

Statistical analyses
The analysis of variance was performed by using the Statgraphics Plus 4.0 and the Principal Components Analysis (PCA) was performed by using the Unscrambler® v.9.2 software (Camo A/S, Oslo, Norway).

5.2 RESULTS AND DISCUSSION

Waxy wheat flours properties and dough leavening performances

The characteristics of Italian waxy wheat flours are summarized in Table 1. As expected, waxy flour contained a significantly lower amount of amylose with respect to non waxy flour. The protein content was in agreement with expectations and resulted higher than that of Aubusson flour (for which the protein content resulted of10.8±0.1 % db) but lower than that of the commercial flour sample which is actually a blend of high bread-making quality flours. Flours from wx118 and BHWX2-2a derive both from wheat of soft type and have a lower ash content with respect to flours from wheats of hard type. Protein quality is generally considered to be closely correlated to dough strength and flour bread-making performances, which can be also predicted by measuring the gluten index (GI) and using various empirical rheological tests including farinograph measurements (Carson & Edwards, 2009).

Results summarized in Table 2 indicate that the IWWL have gave high values for both the GI and the SSV, comparable only with those of not waxy flour Aubusson for which the GI and the SSV resulted of 74±6% and 79±2 mL respectively. The BHWX2-2a sample reported the lowest value of SSV and its GI could not be measured. The effects of waxy flour on the baking quality of dough were also measured using a farinograph. The water absorption of waxy flours resulted significantly higher than that of not waxy flour, whereas their dough stability resulted quite low in agreement with several authors (Morita et al. 2002a , Hung et al. 2006, Qin et al.2009).

Only dough from wx123 showed a stability comparable to that of the non waxy Aubusson flour of medium bread making quality for which the stability resulted of 4.3 minutes. Despite the high protein content showed by waxy flours, their degree of softening resulted highly variable and on average higher than that of non waxy flours. The highest softening values have been observed for the two American waxy wheat samples.

Table 1. Chemical composition of waxy and non waxy flours

Lines		Starch (% d.b.)	Amylose (% d.b.)	Protein (% d.b.)	Lipid (% d.b.)	Ash (% d.b.)
wx70	wx	79.4 ±2.2 c	1.5 ±0.4 a	12.0 ±0.1 a	1.8 ±0.1 b	0.66 ±0.01 cd
wx118	wx	79.5 ±0.8 c	1.4 ±0.1 a	12.9 ±0.1 c	1.8 ±0.2 b	0.38 ±0.04 a
wx123	wx	79.2 ±0.6 bc	1.4 ±0.5 a	13.2 ±0.1 d	1.7 ±0.1 ab	0.55 ±0.01 b
BHWX14-7	wx	76.4 ±1.2 a	1.6 ±0.1 a	13.3 ±0.1 d	1.9 ±0.1 b	0.59 ±0.01 bc
BHWX2-2a	wx	77.0 ±2.2 ab	1.7 ±0.2 a	12.2 ±0.1 b	1.5 ±0.1 a	0.38 ±0.01 a
Comm. Flour	wt	77.5±1.2 abc	26.7 ±0.4 b	13.7 ±0.1 e	1.8 ±0.2 b	0.68 ±0.01 d

Values followed by same letter in the same column are not significantly different (P<0.05).

Table 2. Physical characteristics of waxy flour related to bread-making performances

Lines		SSV (mL)	Gluten index (%)	Farinograph analysis: Water Absorption (%)	Develop. Time (min)	Stability (min)	Softening value (UB)
wx70	wx	78 ±4 b	68±2 b	72.0 cd	4.3 d	3.0 b	106 c
wx118	wx	81 ±1 b	70±2 b	70.6 bc	2.9 bc	2.4 a	136 d
wx123	wx	88 ±2 c	79±5 c	74.3 e	4.8 e	4.1 c	87 b
BHWX14-7	wx	81 ±1 b	44±2 a	73.2 de	3.2 c	2.1 a	158 e
BHWX2-2a	wx	66 ±1 a	-	69.5 b	2.6 b	2.2 a	206 f
Comm. Flour	wt	80 ±1 b	89±2 d	56.6 a	2.2 a	18.8 d	10 a

Values followed by same letter in the same column are not significantly different ($P<0.05$).

The leavening of dough is of key importance for bread-making (Dobraszczyk et al. 2001). The CO_2 produced by yeast during fermentation accumulates in the air cells retained by the dough determining an increase in their size. The analysis of this process during the leavening phase allows predicting the effects of flours' characteristics (e.g. flour origin, dough strength, etc.) on bread quality (Riva et. al, 2004). This analysis was performed by using scanned images of small dough samples because the limited amount of material available did not allow to carry out rheofermentometer measurements.

During the leavening tests (Figure 1) the IWWL achieved a maximum relative increment of dough radial surface (At/At_0) ranging from 3.9 to 4.6 in the time between 120 and 150 min and remained almost constant afterwards; whereas commercial flour achieved the maximum At/At_0 of about 3.7 in the time between 90 and 120 minutes and decreased sensibly afterwards. At/At_0 remained always under 4.0 for American lines BHWX14-7 and BHWX2-2a used as controls.

When considering parameters like the area of gas cells during leavening (Figure 2), tests performed indicate that for waxy lines the maximum of the ratio between cells area and dough total area is about 20% and is achieved between 120 and 150 minutes after leavening start; this ratio remains constant afterwards. The same ratio increases for about 210 minutes and achieves a maximum of about 29% in case of dough from commercial flour. Contrary to what observed for waxy lines, in case of commercial flour the ratio between cell and total area continues increasing also after that the maximum of At/At_0 has been achieved (see Fig. 1 and Fig. 2). This different behavior has been surely amplified by the decrease of the dough area after At/At_0 maximum achievement observed in case of commercial flour and not registered in case of waxy lines (see Fig. 1).

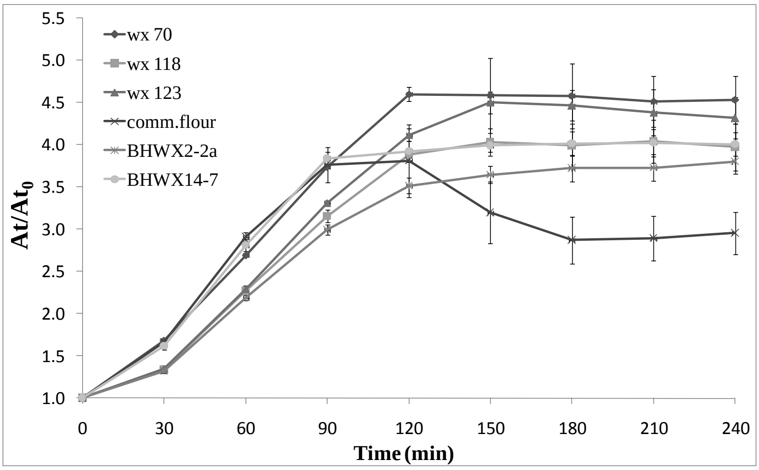

Figure 1. Relative increase of dough radial surface (At/At_0) during leavening of dough confined in Petri dishes

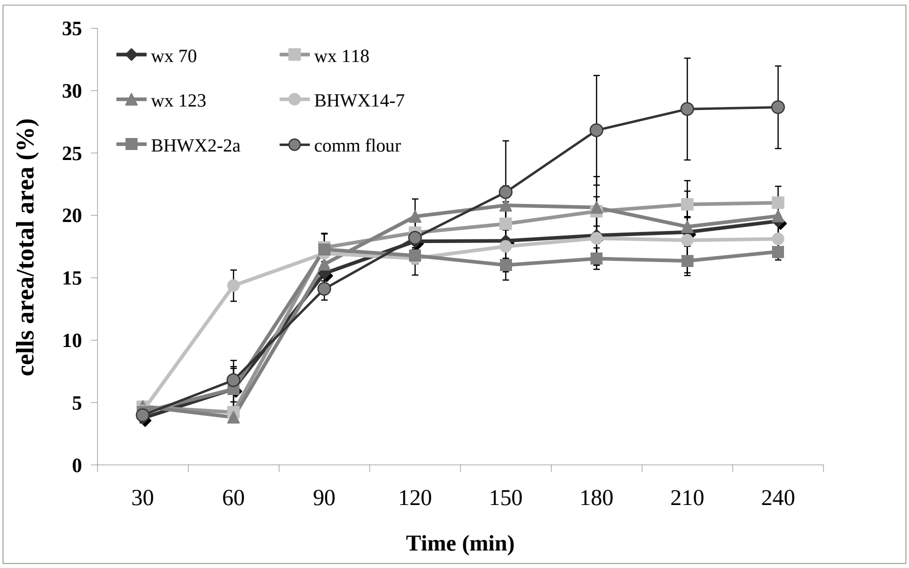

Figure 2. Ratio between cells area and total area of dough during leavening

Figure 3 shows the ratio between cells' area and total cells' area for small, medium and large cells in the time period during which the maximum At/At_0 was achieved (i.e. between 90 and 120 minutes) and at the end of the leavening test (i.e. at 240 minutes). The ratio between medium cells' area and total cells' area increases due to small cells coalescence for all dough samples considered in the time period between 90 and 120 minutes, excepting for dough from BHWX14-7 for which this ratio does not seem to change in this time interval. For wx118, wx123 and commercial flour the medium cells result coalesce into large cells at the end of the leavening time. Percentages of small, medium and large cells range respectively between 94-96%, 3-5% and 0.3-1% for all samples analysed when leavening is concluded.

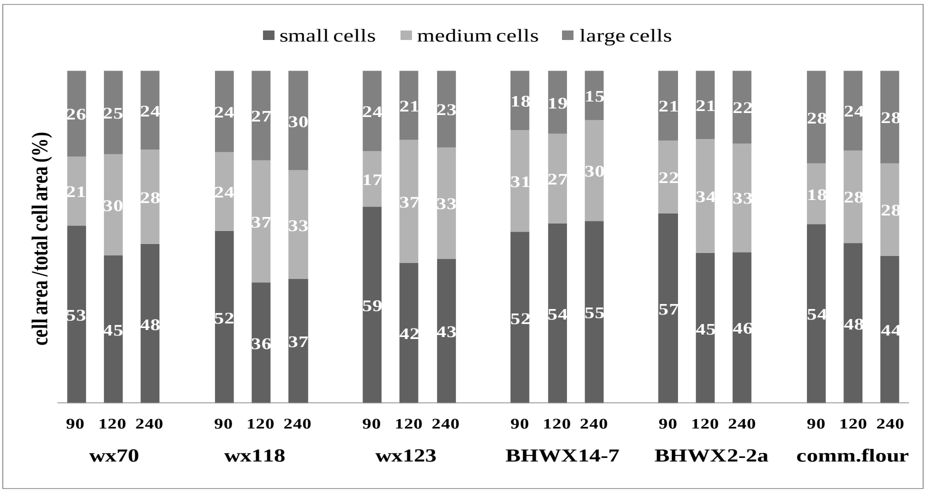

Figure 3. Ratio between dough cell area and dough total cell area at 90, 120 and 240 minutes of leavening (small cell area:0-0.5 mm^2; medium cell area: 0.5-2.5 mm^2; large cell area > 2.5 mm^2).

Bread making properties

Previous studies in the literature indicated that the bread made from waxy wheat has usually slightly larger volume than bread from the non waxy wheat flour. (Morita et al.2002a) The crumb structure of bread becomes typically more porous as the amylose content of flour decreases; as suggested by Hung et al. (2006) amylopectin seems to be more susceptible to α-amylase during fermentation assuring a higher sugar fermentation and therefore a higher gas production in waxy wheat flour as compared with non waxy wheat flour. In case of the Italian samples the low dough stability observed during farinograph test in principle could have been responsible for low volume of corresponding breads. Nevertheless the IWWL produced quite good results. As showed in Table 3 the Italian waxy breads resulted higher than American breads on average; the wx123 bread presented a volume 28% higher than the BHWX2-2a bread. The bread volume of IWWL ranged from 208 to 226 mL and resulted significantly higher than that of controls. The specific volume of breads from IWWL resulted consequently significantly higher than that of controls with wx123 line achieving the highest volume. Table 3 also indicates that waxy wheats generally have significantly higher baking loss than not waxy wheats.

Concerning bread crust color, data in Table 3 indicate that breads from IWWL had lower lightness (L*), redness (a*) and yellowness (b*) with respect to commercial flour breads. Samples from BHWX14-7 wheat seemed to have a crust color comparable to that of IWWL. Concerning bread crumb color, waxy wheats lightness (L*) was comparable for all waxy samples and was significantly lower with respect to commercial flour bread lightness. Greenness (a*) and yellowness (b*) of bread crumb was quite comparable for all wheats considered, with wx118 bread crumb having a yellowness significantly lower than that of commercial flower.

Table 3. Characteristics of Italian waxy bread.

	Italian waxy wheat lines			waxy wheat		not waxy wheat
Baking Test	**wx70**	**wx118**	**wx123**	**BHWX14-7**	**BHWX2-2a**	**Comm. Flour**
Bread Volume (mL)	208 ±3 d	219 ±9 e	226 ±3 e	185 ±6 c	163 ±3 a	173 ±6 b
Weight (g)	32.7 ±0.6 ab	33.1 ±0.6 c	33.0 ±0.3 c	32.8 ±0.8 ab	32.4 ±0.8 ab	31.8 ±0.2 a
Baking loss (%)	26.6 ±1.6 bc	26.3 ±0.8 b	28.0 ±1.1 d	27.8 ±1.3 cd	27.7 ±1.5 cd	23.7 ±0.5 a
Height (mm)	73.2 ±3.7 bc	77.7 ±1.6 d	81.1 ±2.0 e	76.4 ±3.7 cd	63.6 ±3.4 a	71.2 ±1.7 b
Specific Volume (mL/g)	6.37 ±0.11 d	6.62 ±0.19 e	6.89 ±0.08 f	5.66 ±0.14 c	5.08 ±0.15 a	5.41 ±0.19 b
Bread crust color						
L*	43.84 ±2.63 a	44.14 ±1.11 a	47.94 ±2.59 b	44.05 ±4.40 a	53.71 ±1.99 c	52.35 ±1.39 c
a*	9.55 ±1.21 a	9.37 ±0.59 b	8.56 ±0.97 b	8.42 ±0.46 b	6.15 ±1.23 b	10.81 ±0.72 c
b*	24.69±3.06 ab	24.36 ±0.95 a	27.30 ±1.22 bc	24.13 ±2.81 a	28.83 ±2.04 c	32.73 ±1.68 d
Bread crumb color						
L*	49.57 ±3.65 a	44.17 ±7.22 a	49.41 ±5.43 a	44.29 ±11.76 a	43.05 ±6.68 a	64.92 ±2.16 b
a*	-1.82 ±0.17 a	-1.51 ±0.57 a	-1.79 ±0.37 a	-1.70 ±0.87 a	-1.34 ±0.30 a	-1.54 ±0.14 a
b*	10.39 ±2.24 ab	8.59 ±1.75 a	9.09 ±2.43 ab	11.71 ±2.41 b	10.19 ±1.90 ab	11.25 ±1.06 b

Values followed by same letter in the same column are not significantly different ($P<0.05$).

Although the specific volume of breads from IWWL resulted significantly higher than those of controls, the image analysis of bread slices showed no significant differences in the ratio between cell area and total area of IWWL and commercial flour breads (Table 4). This ratio seems to be lower for slices from American samples. The results of the present study indicated also that the number of large cells does not vary significantly among waxy and commercial flour breads (Fig. 4), that the medium cell mean area is comparable in waxy and not waxy samples and that the large cell mean area is significantly higher for wx118 and wx123 samples with respect to non-waxy samples (Table 4). These results seem to be in a quite good agreement with information available in the literature (Morita et al. 2002, Lee et al. 2001, Hayakawa et al. 2004) indicating that breads from waxy wheats are characterized by very large cells.

Table 4. Bread cell characterization

	Italian waxy wheat lines			waxy wheat		not waxy wheat
	wx70	wx118	wx123	BHWX14-7	BHWX2-2a	Comm. Flour
cell area/total area (%)	36.01± 2.28 b	35.83 ± 1.80 b	35.05 ± 1.13 b	31.67 ± 1.68 a	31.69 ± 1.02 a	34.44 ± 1.60 b
small cell total area (mm^2)	96.12 ± 9.56 a	95.76 ± 12.62 a	101.80 ± 7.42 ab	111.11 ± 12.26 c	107.21 ± 17.55 ab	135.22 ±12.58 d
medium cell total area (mm^2)	130.98 ± 14.72 a	128.82 ± 16.50 a	135.67 ± 24.89 a	137.21 ± 16.73 a	132.61 ± 7.94 a	177.56 ± 29.25 b
large cell total area (mm^2)	629.97 ± 60.29 ab	608.22 ± 64.48 ab	635.87 ± 56.60 ab	663.39± 84.49 a	570.36 ± 50.71 b	591.42 ± 58.81 b
small cell mean area (mm^2)	0.06 ± 0.01 c	0.06 ±0.01 bc	0.06 ± 0.01 bc	0.06 ± 0.01 b	0.05 ± 0.01 a	0.07 ± 0.01 d
medium cell mean area (mm^2)	1.18 ± 0.03 a	1.15 ± 0.08 a	1.15 ± 0.10 a	1.14 ± 0.06 a	1.17 ± 0.05 a	1.13 ± 0.07 a
large cell mean area (mm^2)	18.28 ± 4.73 abc	20.28 ± 0.60 c	19.68 ± 3.07 bc	18.41 ± 3.91abc	15.98 ± 2.96 ab	14.41 ± 2.29 a

Values followed by same letter in the same column are not significantly different (P<0.05).

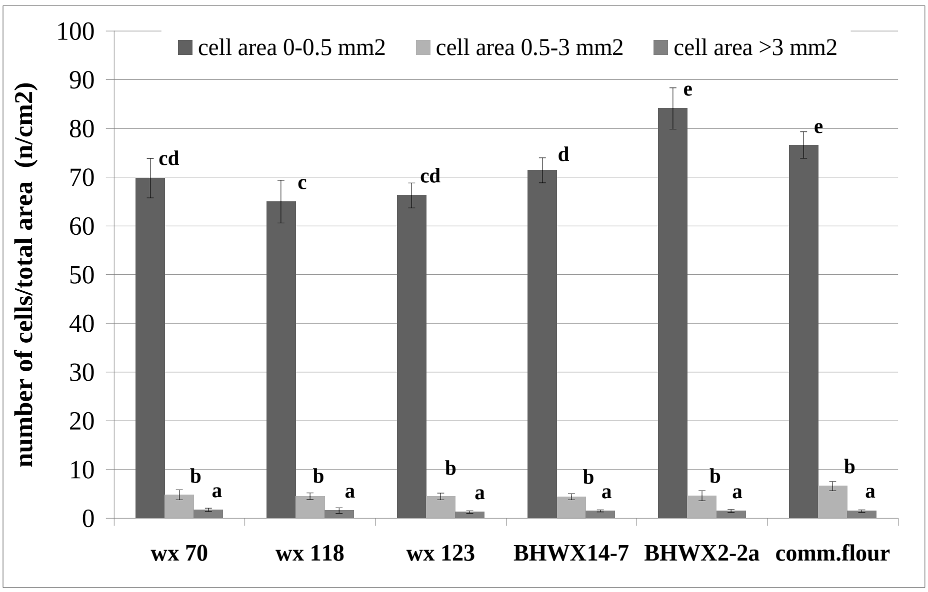

Figure 4. Bread cell density for small, medium and large cells

In order to asses bread staling, bread crumb moisture, water activity and firmness have been analysed on day 0, 1, 3 and 7 after baking.

Concerning bread crumb moisture, loaves from wx118 have higher crumb moisture with respect to samples from commercial flour on day 0 (Fig. 5). This difference disappears on day 3 and only loaves from wx123 have higher crumb moisture with respect to commercial flour on day 7.

Data indicated that the higher crumb moisture loss takes place between day 0 and day 1 for all loaves from waxy wheats.

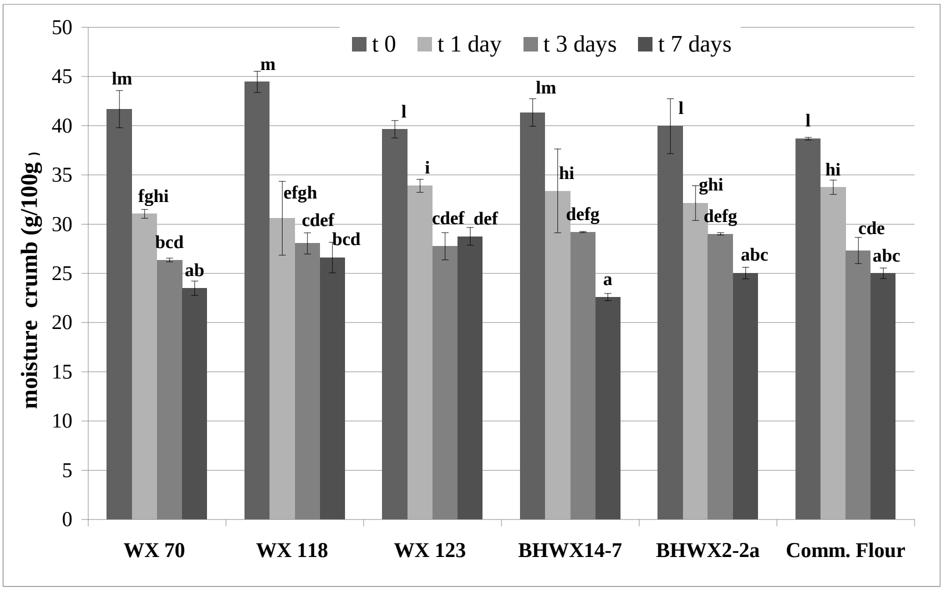

Figure 5. Moisture crumb of loaves on day 0, 1, 3 and 7 after baking.

Figure 6 indicates that crumb water activity is around 0.96 for all loaves considered at time T_0. This value decreased by about 2% and 4% respectively on day 1 and 3 with respect to day 0 for all loaves. On day 7 the water activity of most of the loaves from waxy wheats was comparable to that of commercial flour, including loaves from wx123 which resulted having a higher crumb moisture. On this day only loaves from wx70 and BHWX14-7 resulted having water activity levels significantly lower.

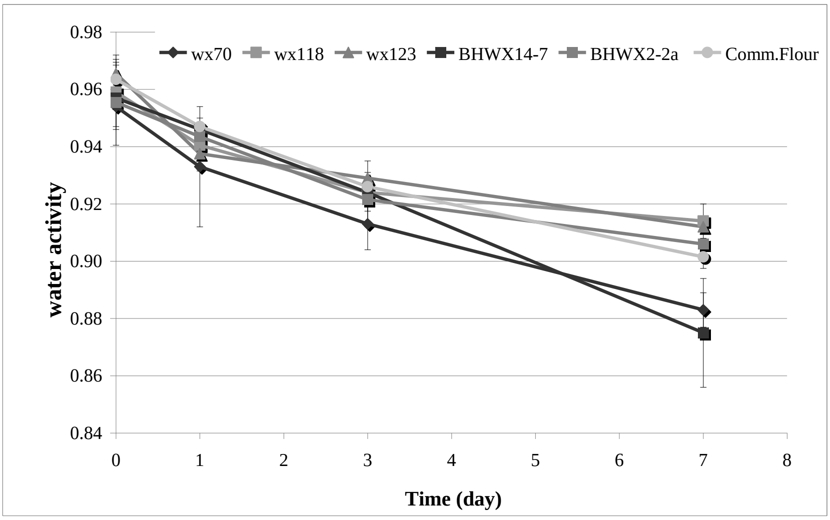

Figure 6. Water activity of loaves on day 0, 1, 3 and 7 after baking

Considering that the texture of bread is assumed to be one of the most important factors determining taste, breadcrumb firmness was finally analysed. For all waxy wheats this parameter resulted lower than that of commercial flour. This trend was observed for each storage period with the exception of BHWX2-2a whose breadcrumb achieved on day 3 the values registered for commercial flour breadcrumb firmness on day 7 (Figure 7).

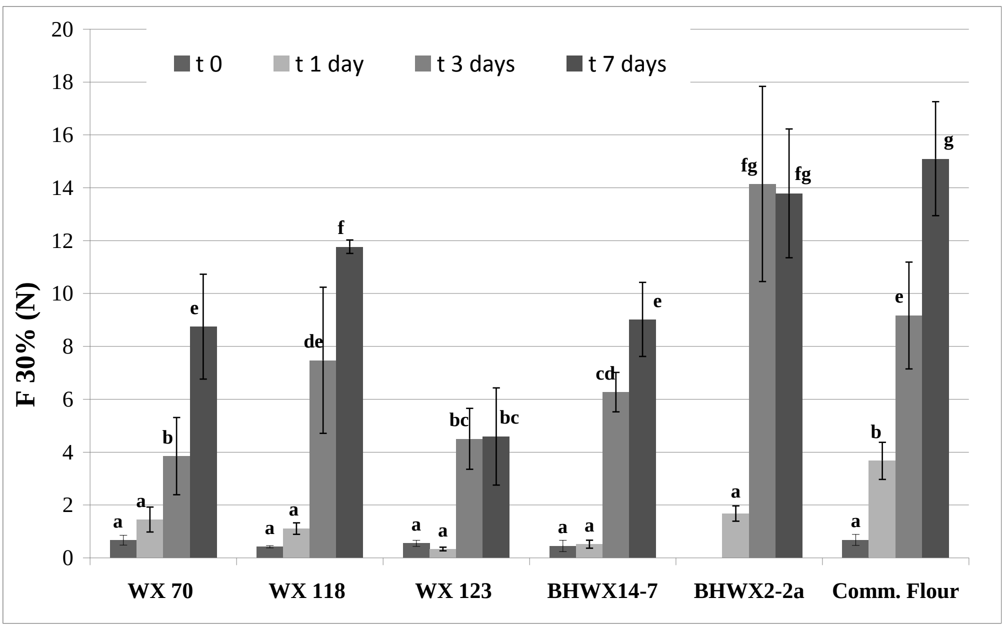

Figure 7. Firmness of loaves on day 0, 1, 3 and 7 after baking.

The breadcrumb from wx123 resulted having the lowest firmness on day 7. This result could be partly related to the higher moisture observed on day 7. As mentioned above wx123 breadcrumb resulted indeed having higher moisture with respect to commercial flour breadcrumb on day 7. Nevertheless wx123 breadcrumb moisture resulted only about 3% over commercial flour crumb moisture and this difference can be hardly put in correlation with wx123 breadcrumb delayed firming. Indeed breadcrumbs from other waxy lines (e.g. wx70) resulted having a delayed firming, but their moisture content resulted comparable to that of commercial flour. On the other hand, a possible correlation between breadcrumb moisture content and delayed firming is still debated in the literature. In fact some studies apparently confirm the presence of a direct correlation (e.g. Ghiasi et al. 1984, Hayakawa et al. 2004, Lee et al. 2001; Morita et al., 2002b), whereas others (e.g. Bhattacharya et al. 2002) conclude that delayed firming is not moisture related.

A PCA model was made including the three IWWLs selected, the two American samples and the commercial flour (6 samples in total) and 37 variables related to flour chemical composition and technological performances of dough and breads were considered in order to address differences and similarities between samples. Values of parameters related to bread staling have been measured on day 7 after baking (Figure 8). The explained variances on PC1 and PC2 account for 67% of variance. As regards the PC1, the variability is related to parameters positively associated with farinograph stability, amylose content, bread small and medium cells density, bread small and medium cells total area, breadcrumb firmness (F 30%), crumb L* and b* and is related to parameters negatively associated to bread weight, water absorption farinograph, bread baking loss, ratio between dough medium cells area and total cells area, bread volume, mean large cells area, farinograph development time. As regards the PC2, the variability is related to parameters positively associated to farinograph softening, ratio between bread small cells area and total cells area, crumb a* and is related to parameters negatively associated to bread volume, SSV, bread height, ratio between bread cells area and total area, Gluten Index (GI), crust a* and small cells mean area. The score plot shows that commercial flour is positioned on the right in the plane area whereas on the left in the plane there are the IWWLs. This plot shows also that bread from BHWX2-2a, which resulted of the lowest quality, differ considerably from IWWLs.

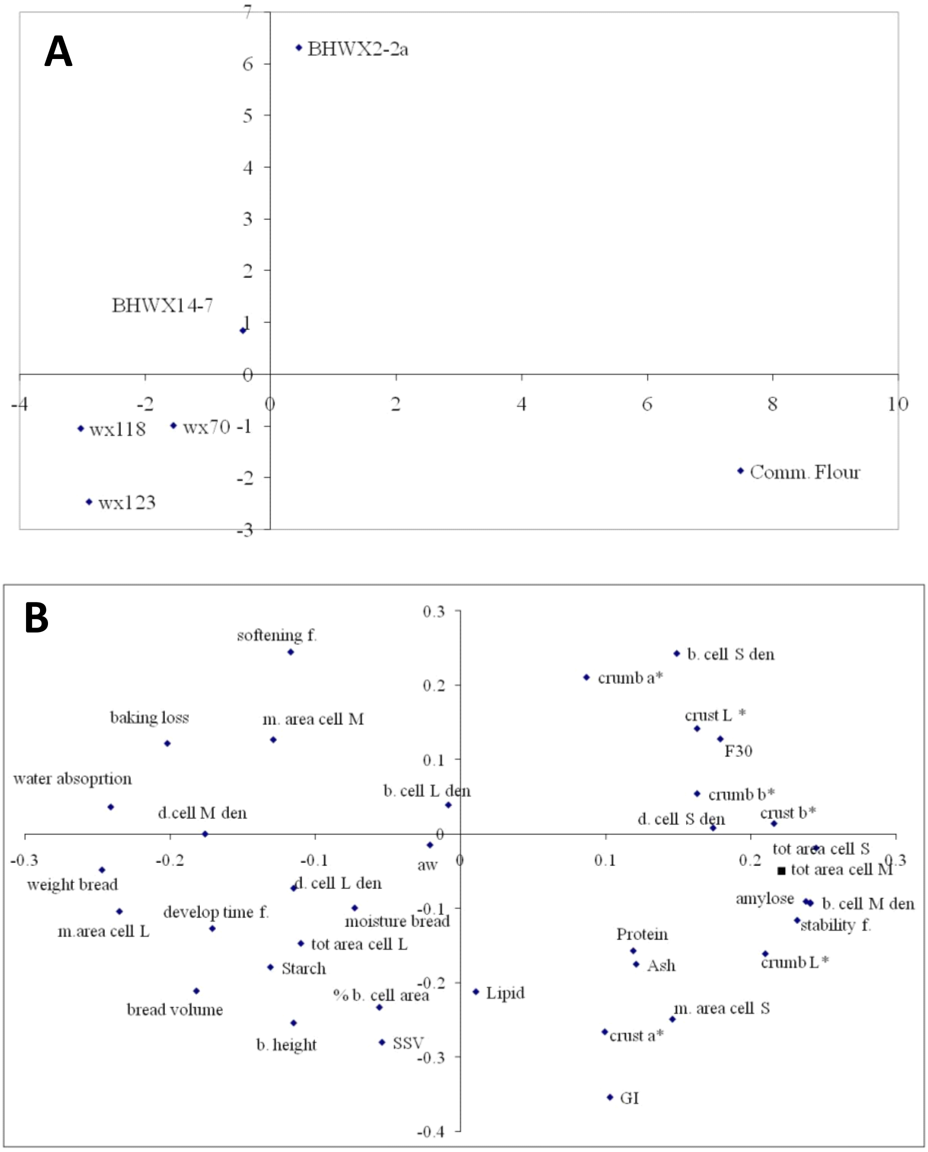

Figure 8. Score Plot (A) and Loading Plot (B) of the PCA performed on the waxy samples and commercial flour (PC1:41%; PC2: 26%) Bread crumb moisture, bread crumb aw and F30% were measured at 7 days after baking dough. Acronyms used in the Loading plot refer to the ratio between small, medium, large cells area and total area of *dough* (d. cell S den, d. cell M den, d. cell L den as measured after 120 minutes during the leavening test), density of *bread* small, medium, large cells (b. cell S den, b. cell M den, b. cell L den), bread S, M and L cells mean area (m. area cell S, M, L) and S, M and L cells total area (tot area cell S, M, L), ratio between cells area and total area of bread (% b. cell area).

5.4 CONCLUSIONS

Bread making characteristics and staling resistance of three IWWLs have been analysed in this work. The specific volume of breads from IWWLs resulted significantly higher than that of controls and the estimated mean area for the large cells of breads resulted significantly higher for most of the IWWL samples with respect to non-waxy samples.

Concerning bread color, the results obtained indicate that breads from IWWL have crusts with lower lightness (L*), redness (a*) and yellowness (b*), whereas their breadcrumbs have lower lightness (L*) with respect to commercial flour breads.

As expected breadcrumb firmness of all breads from IWWLs resulted lower than commercial flour breadcrumb firmness for at least 7 days after baking.

Among IWWLs considered, bread from wx123 line achieved the highest volume. Its breadcrumb resulted having the lowest firmness and breadcrumb moisture was about 3% over commercial flour breadcrumb moisture on day 7 after baking. Despite existing literature (e.g. Bhattacharya et al. 2002) maintains that delayed firming can be hardly explained only in terms of higher breadcrumb moisture, it is worth noticing that the relative moisture decrease of wx123 breads during 7 days of storage resulted of 27% against a relative moisture decrease of 35% registered during the same period for breads from commercial flour. However, according to Schiraldi et al. (1996) bread firmness can be related to the presence of water binding biopolymers and it seems hence reasonable to conclude that the different relative decrease observed in bread moisture could actually explain the lower bread firmness detected for wx123 breads. Indeed the relative decrease observed in water activity during the 7 days storage period (6%) did not change between wx123 and commercial flour breads. This allows concluding that the lower relative decrease in wx123 breads moisture has to be attributed to a lower decrease in water binding biopolymers in these breads. In conclusion the waxy wheat lines adapted to the Italian environmental conditions considered in this study show on average better bread-making qualities with respect to American standard waxy lines.

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6. INFLUENCE OF FLOUR BLENDS ADDED WITH ITALIAN WAXY WHEAT LINE ON BREAD MAKING QUALITY AND STALING KINETICS

6.1 STATE OF THE ART

The employment of waxy wheat for bakery products has been widely studied because this raw material can retard staling and extend shelf-life of breads (Bhattacharya et al. 2002, Ghiasi et al. 1984, Hayakawa et al. 2004, Lee et al. 2001, Morita et al. 2002b). These effects seem to be due to the low amylose content of waxy flour which seems to retard starch retrogradation, one of the major causes of bread staling. Some studies (Lee et al. 2001, Morita 2002a, Baik and Lee 2003; Guo et al. 2003a, Hayakawa et al. 2004, Takata et al. 2005; Sahlstrom et al. 2006) also report that, although producing breads of higher volumes, waxy flour is characterized by inferior functional properties; in fact dough from waxy wheat has high stickiness, difficult machinability and results in breads of unacceptable appearance with a more porous crumb structure. For these reasons, various research studies have tried to use waxy wheat as a source of blending flour in order to find the optimum amylose/amylopectin ratio for producing end-use products of good quality. Lee et al. 2001 and Hayakawa et al. 2004 showed that the incorporation of 20% waxy wheat flour (WWF) produced considerable improvement in shelf-life characteristics, whereas the increase on amount of WWF substitution up to 50% had lower loaf volume, more porous crumb structure and higher level of starch retrogradation than regular wheat. When the waxy flour additions were below 25%, the firmness of Chinese steamed bread stored at -18°C for 3 days gradually decreased with the increase of waxy flour in Qin *et al*. 2007. Pham et al. 2007 found that bread from waxy wheat flour is significantly softer than the commercial white flour one during storage and the qualities of breads are also improved using partial waxy wheat flour substitution (10, 30 or 50%) for commercial white flour. Qin et al. 2009 found finally that waxy wheat flour blend at 15% was optimal in retarding staling without significantly decreasing fresh bread quality.

Whereas previous studies aimed at using waxy flour just as additive for retarding staling of breads from common wheat flour, the present study adopts an opposite approach. Flour blends incorporating increasing shares of commercial flour have been prepared starting from flours obtained from completely waxy wheat lines suitable for cultivation in Italy which resulted having better bread-making qualities compared to American waxy wheat lines used as controls. Better qualities consisted in a higher bread volume, in a higher breadcrumb softness seven days after baking and in a breadcrumb cell size not as high as that usually reported in the literature for waxy wheat breads (see chapter 5). Nevertheless, flour from Italian waxy wheat lines (IWWL) gave dough with relevant stickiness, The present work was then aimed at investigating the possibility of reducing this negative property by using increasing percentages of commercial non-waxy wheat flour with high bread making quality in IWWL flours.

6.2 MATERIALS AND METHODS

Materials

Three Italian waxy wheat lines (IWWL) have been used for the analysis: wx70, wx 118 and wx123 respectively hard, soft and medium type. Two homozygous waxy wheat (WQL6K107-BHWX14-7, PI 612546, hard type, and WQL6K107-BHWX2-2a, PI 612545, soft type), kindly provided by C. Morris (Washington State Univ., Pullman, WA) (Morris & Konzak, 2001) and a non-waxy commercial flour of high bread-making quality were used as control for all technological analyses. The non-waxy commercial flour was added at levels of 0%, 20% and 40% to flours from the above mentioned waxy wheats in order to create flour blends respectively with a 100%, 80% and 60% of waxy flours. Bread-making qualities and bread staling resistance of blends have been then analysed and compared.

Methods

The Italian waxy wheat lines considered were grown in St. Angelo L. (Italy) during the 2009-10 growing season.
The chemical proximate composition was determined on flour prepared by milling the grain with a Bona Quadrumat Labormill (Bona, Monza, Italy). Protein contents (N x 5.7 dry weight, AACC 39-10, AACC International, 2000) were determinate by the NIR System Model 6500 (Foss NIR Systems, Laurel, MD).
The rheological evaluation was performed by a Brabender Farinograph (Brabender OHG, Duisburg, Germany) using a 50g mixer according to the ICC 115-D (ICC 1992).

Pasting properties
Flour pasting properties were determined using a Rapid Visco Analyzer (RVA-4 model, Newport Scientific, Sidney, Australia) according to ICC162. Flour samples of 3.5 g were dispersed in 25 mL of distilled water, scaling both sample and water weight on a 14% flour moisture basis. The suspensions were subject to the following temperature profile: holding the sample at 50°C for 1 min followed by heating the sample from 50 to 95°C in 3.75 min, holding the sample at 95°C for 2.5 min, cooling the sample back to 50°C in 3.75 min, and holding the sample at 50°C for 2 min. The following indices were considered: pasting temperature (PT, °C at which an initial increase in viscosity occurs), peak viscosity (PV, Pa·s; maximum paste viscosity achieved during the heating cycle), breakdown (BD, Pa·s, index of viscosity decrease during the first holding period, corresponding to the peak viscosity minus the viscosity after the holding period at 95°C), final viscosity (FV, Pa·s; paste viscosity achieved at the end of the cooling cycle), setback (SB, Pa·s; index of the viscosity increase during cooling, corresponding to the difference between the final viscosity and the viscosity reached after the first holding period) using Thermocline for Windows 3 6 (TCW3) software provided with the RVA. Measurements were performed in triplicate and the average value was used.

Gel texture analysis
The texture of the gel formed after heating the slurry of wheat flour-water is central to its functionality and is a fairly accurate indicator of how a wheat sample would influence the final product (Bhattacharya et al., 2002).
After RVA cycle, the stirring paddle was discarded and the meal past was split into three small parts (7.0 g of gel each) enclosed into as many vessels kept at 4°C for two weeks.

The gel formed was subjected to texture profile analysis using a Texture Analyzer TA-HD- plus (Stable Micro System Ltd, UK) on days 0, 1, 3, 6 and 14. The RVA gel firmness was assessed based on the maximum force required to penetrate the gel with a cylindrical probe (4 mm diameter) up to 30% of gel height.

Mixolab test

Dough rheological properties were investigated by using mixolab (Chopin, Triplette et Renaud, Paris, France); this instrument allows mixing and simultaneous heating or cooling of the dough under controlled temperature. Required amount of flour for analysis was calculated by Mixolab software according to input values of flour moisture and water absorption. The water absorption was calculated as the water absorption of flour obtained, during Farinograph test, at a optimal consistence of 500 BU. The settings used in the test were 20 minutes for mixing at 30°C, temperature increase at 4°C/min until 90°C, 7 minutes holding at 90°C, temperature decrease at 4°C/min until 50°C and 5 minutes holding at 50°C. The mixing speed during the entire assay was 80 rpm. All the measurements were performed in duplicate and the average value was used.

Dough stickiness

The stickiness of the dough produced by farinograph was determined by using a Texture Analyzer TA-HD-plus (Stable Micro System Ltd, UK). The stickiness, also known as adhesiveness, of the dough (15 g of weight each) was evaluated as the negative area of a force-time curve measured during force removal; the time of plate detachment (diameter: 35 mm) from the sample was also considered after a 5 s compression by a force of about 3 N. This plate was raised at a speed of 1 mm/s after compression.

Rheological properties

Viscoelastic properties of dough prepared from waxy and non-waxy flours and related blends were studied by dynamic oscillatory measurements performed on a Physica MCR100 Rheometer (Physica Messtechnic GmbH, Ostfildern, Germany).

The dough was obtained by mixing (1 minute) in the Glutomatic 2200 (Perten Instruments, Stockholm, Sweden) 10g of flour and amounts of water in the same proportions considered in the farinograph analysis. A parallel plate geometry (25 mm diameter, 1 mm gap) was used, with corrugated plates to prevent dough slippage. The temperature was regulated at 25 °C by a circulating bath and Peltier-based temperature control. After loading, the excess dough was trimmed and a thin layer of paraffin oil was applied to the edge of the exposed sample to prevent moisture loss during measurements. The dough was allowed to rest for 30 min in order to equilibrate stresses, before starting the test. The following tests were performed on each sample: strain sweep tests, in the range of 0.01–200%, at 1 Hz frequency; frequency sweep tests, in the range of 0.1–10 Hz, at 0.03% strain. Each test was performed at least three times. Data were analyzed using US200/32 v.2.50 rheometer software (Physica Messtechnic GmbH, Ostfildern, Germany).

Bread making procedures

Bread loaves from 25 g of flour were produced according to Method 10-10B (AACC International, 2000) with some modifications (e.g. absence of milk and potassium bromated), introduced to make the method more similar to the one traditionally used in Italy (Borghi et al., 1996). The baking formula (flour basis) was flour 50.0g (14%db), sugar 2.0 g, salt 1.0 g, shortening 1.5 g, yeast 2.0 g and 2 ppm of ascorbic acid. The water added to form optimum dough was calculated from the water absorption of the flour during a Farinograph test performed to obtain an optimal consistence of 500 BU. All ingredients were mixed for 3 min. The dough was divided into two pieces (about 45 g/piece) and then fermented in cabinet at

30°C at a relative humidity of 80% for 150 min. The punching was done two times during the fermentation (after 50 min and after 75 min). All dough samples were placed in apposite baking pans (4.2 x 7.0 cm in top, 3.1 x 6.0 cm in bottom, and 4.0 cm in depth) and fermented for 70 min before baking. Dough pieces were baked at 220°C for 20 min. Bread loaves were allowed to cool for a minimum of 60 min before further test. Baking losses were estimated as the percentage variation of dough weight before and after baking.

Bread volume was measured by rapeseed displacement. A commercial flour of high bread-making quality was used as control for all technological analyses.

Bread quality evaluations

The volume of each loaf was measured by rapeseed displacement. For the staling study, the loaves were placed in perforated plastic bags (15x20 cm), which were subsequently sealed and stored at 22°C.

On day 1 , day 3 and day 7 after baking, two loaves of each formulation were sliced into 2.5 cm thick slices. The two slices from the middle were further analyzed. Characteristics of bread crumb were determined by using a scanner (Epson Perfection 3170 Photo, Saiko-Epson Corporation, Japan) and the image analysis instrument used for the leaving test. Slice image and data on number of gas cells and cells' area were obtained. The total number of cells, the number of cells smaller than 0.5 mm^2, the number of cells larger than 3.0 mm^2 and the number of cells with a surface between 0.5 and 3.0 mm^2.

Moreover firmness, water activity and moisture of every loaf crumb were evaluated.

Color analysis

A color meter (CR 210, Minolta Co., Osaka, Japan) was used to measure the lightness and saturation of the color intensity value of bread utilizing the CIE-LAB uniform color space procedure. CIE-Lab-System color value L*, a* and b* as measures of lightness, redness-greenness, and yellowness-blueness, respectively, were recorded for each sample and compared with a white standard. Each measurement was replicated five times and the average value was used.

Moisture content and water activity

The moisture of bread crumb and of the bread were determined according to the official standard methods AACC 44-15 (2001). The water activity of bread crumb was determined by LabMaster-aw (Novasina AG, Lachen, Switzerland) at 25°C and has been measured after 5 min of stability in the detected water activity. Each measurement was replicated five times and the average value was used.

Bread texture analysis

Firmness was measured by a modified AACC 74-09 method (AACC International, 2000). Bread slices were tested using a Texture Analyzer TA-HD- plus (Stable Micro System Ltd, UK) with a 36 mm cylindrical probe. The firmness of the bread samples was evaluated as the force required to compress the bread slices (25 mm thick) with a cylindrical probe up to 30% of deformation. Firmness values reported were the average of four measurements.

Statistical analyses

The analysis of variance was performed by using the Statgraphics Plus 4.0.

Least significant difference (l.s.d. p=0.05) was used to compare mean value within the waxy class.

6.3 RESULTS AND DISCUSSION

Flours and dough properties

As known waxy flours play an important role in retarding bread staling. Nevertheless various studies (Zhao et al. 2009) already highlighted that the presence of waxy wheat increases dough stickiness with respect to non-waxy wheat (Yi et al.2009, Hung et al. 2005, Morita et al. 2002a). This is confirmed also for the three Italian waxy lines and the related controls considered in this study as summarized in Table 1.

Table 1. Adhesiveness of dough from waxy flour blends with commercial flour

	Composition of blends		Adhesiveness test	
Lines	waxy flour (g/100 g)	commercial flour (g/100 g)	Stickiness (Nmm)	Time of plate detachment (s)
wx70	100	0	130.9 ab	19.6 gh
	80	20	113.9 bc	10.9 d
	60	40	90.0 bc	9.5 cd
wx118	100	0	126.6 ab	17.1 efg
	80	20	84.8 ef	11.6 d
	60	40	58.6 fg	8.2 c
wx123	100	0	114.5 bc	16.1 ef
	80	20	96.6 cde	11.6 d
	60	40	41.9 g	5.4 b
BHWX14-7	100	0	95.0 cde	20.7 h
	80	20	138.0 a	18.0 fg
	60	40	136.4 ab	15.1 e
BHWX2-2a	100	0	88.2 e	21.7 h
	80	20	112.4 bcd	21.3 h
	60	40	130.7 ab	17.1 efg
Comm. Flour	0	100	17.9 h	2.3 a

Values followed by same letter in the same column are not significantly different ($P<0.05$).

On the contrary,, dough from the commercial flour used in this study is characterized by a lower stickiness and much lower plate detachment time with respect to dough from completely waxy flours. Table 1 also shows that plate detachment times decrease significantly for most of the dough samples from flours blends with 80% and 60% of waxy flours. Concerning dough stickiness, data reported in Table 1 indicate that this index decreases *only* in case of dough samples from flour blends made of IWWLs with respect to dough samples from completely waxy flours. In particular the stickiness of dough samples from flour blends with 80% of waxy flours are not always significantly different from 60% blends, whereas the stickiness of dough samples from flour blends with 60% of waxy flour is always significantly different from completely waxy flours. The increase of commercial flour amount in the flour blends considered increases flour blends protein content moderately and dough internal forces and strength markedly (see also farinograph stability in Table 2).

Table 2 also shows how the addition of commercial flour positively affects some parameters characterizing flour blends quality. Flour water absorption results reduced due to the increase in amylose content. Overall, the very high stability of the commercial flour used improves waxy dough samples stability and blends with commercial flour result characterized by higher farinograph stability. Also dough softening, indicating the dough weakening during kneading, is reduced in agreement with Qin et al. (2009).

Table 2. Protein content and farinograph parameters of Italian waxy flours blends

		Composition of blends		Farinograph analysis			
Lines		waxy flour (g/100 g)	Protein content (% d.b.)	Water Absorption (%)	Develop. Time (min)	Stability (min)	Softening value (UB)
wx 70	wx	100	12.0	72.0	4.3	3.0	106
		80	12.1	68.1	4.9	4.5	79
		60	12.6	65.3	4.5	5.6	67
wx 118	wx	100	12.9	70.6	2.9	2.4	136
		80	12.9	66.7	4.1	3.8	111
		60	13.0	60.0	4.2	3.8	93
wx 123	wx	100	13.2	74.3	4.8	4.1	87
		80	13.3	70.3	5.8	6.9	69
		60	13.4	66.9	4.4	7.0	67
BHWX14-7	wx	100	13.3	73.2	3.2	2.1	158
		80	13.2	69.0	4.3	3.1	122
		60	13.4	66.8	3.8	4.3	87
BHWX2-2a	wx	100	12.1	69.5	2.6	2.2	206
		80	12.2	67.2	2.6	2.4	204
		60	12.6	65.3	3.3	3.2	131
Comm. Flour	wt	0	13.7	56.6	2.2	18.8	10

Properties of starch in the flour blends were deduced from RVA profiles. The RVA profiles can be considered a reflection of the granular changes that occur during starch gelatinization and retrogradation (Mariotti et al. 2005). Viscosity profiles obtained from two IWWL flours and from BHWX2-2a flour with related blends are plotted under Figure 1. The first plot in this figure includes also the viscosity profile obtained from the commercial non-waxy flour. As showed in these plots 100% waxy flours result having lower gelatinization temperature (around 80 °C), higher peak viscosity, higher breakdown and lower setback whit respect to non-waxy wheat flour in agreement with Qin et al. 2009 and Bhattacharya et al. 2002. When considering flour blends, these plots show that peak viscosity decreases by 22-28% in case of blends with 80% of waxy flour and by 46-54% in case of blends with 60% of waxy flour with respect to completely waxy flours. Breakdown of flour blends decreases, setback increases and peak time increases with respect to profiles of 100% waxy flours in agreement with Bhattacharya et al. 2002. These behaviors can be explained by considering that the commercial flour used in blends has lower peak viscosity, lower breakdown, higher setback and higher peak time.

RVA profiles of flour blends show also a further lower peak viscosity (more pronounced in case of blends with 60% of waxy flour) at a peak time corresponding to the peak time of commercial flour. This second viscosity peak is due to the presence of non-waxy starch granules in the flour blends. The presence of these granules does not seem instead to affect the final viscosity of flour blends which results not significantly different from that of 100% waxy flour. This indicates that the high resistance to retrogradation observed in the samples with waxy starches is maintained also in the samples related to flour blends.

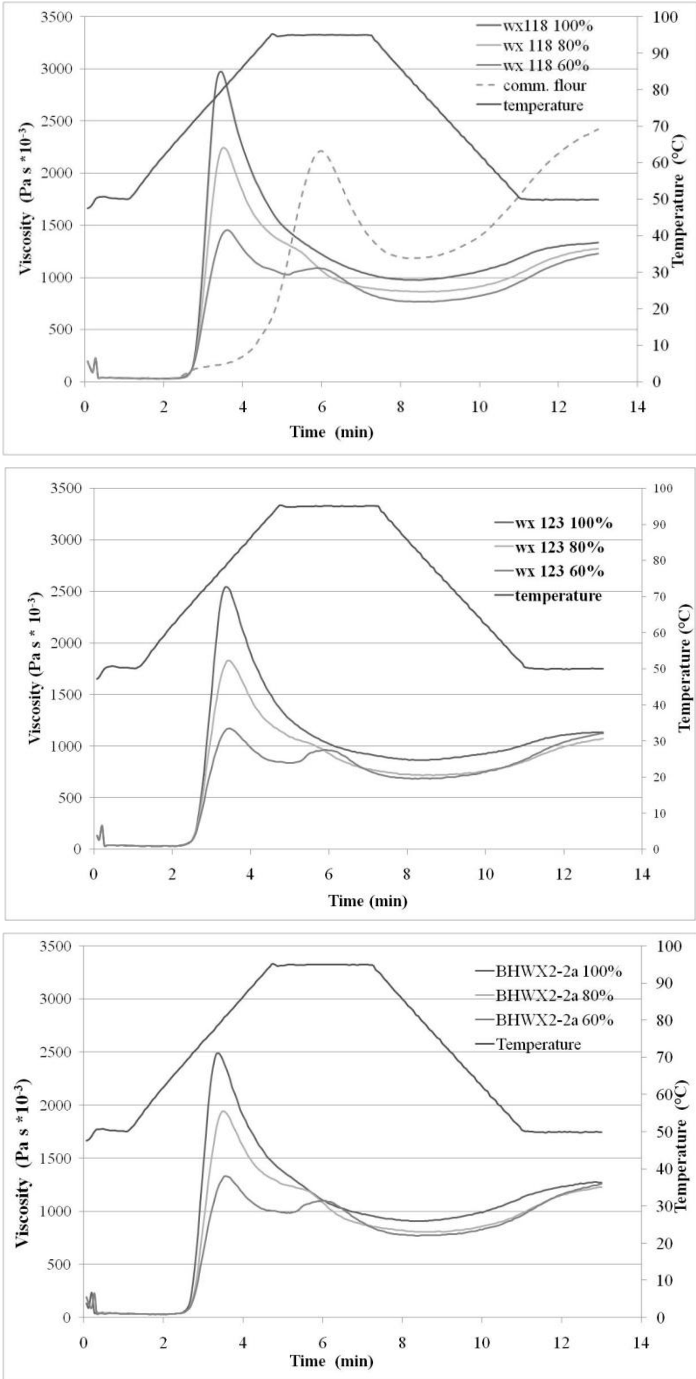


Figure 1. RVA profiles of flours from wx118, wx123, BHWX2-2a and related blends.

In agreement with Bhattacharya et al. 2002 waxy flours and their blends with commercial flour do not form a firm gel (see Figure 2). Gel firmness did not increase during storage at 4 °C for 14 days and ranged between 1.8 and 2.7 $N\cdot10^{-2}$ after 6 days of storage, whereas firmness of gel from pure commercial flour was about 15 times higher (i.e. about 36 $N\cdot10^{-2}$) after a same storage time. This could indicate that the low staling rate observed for breads from waxy flours will be confirmed also for breads from blends of waxy and non-waxy wheat flours. Italian and American waxy gels showed a similar firmness during the time period considered. The decrease observed in the commercial flour gel firmness on day 14 is probably due to the enzymatic action of micro-organisms which could develop because the storage was not realized in completely aseptic conditions.

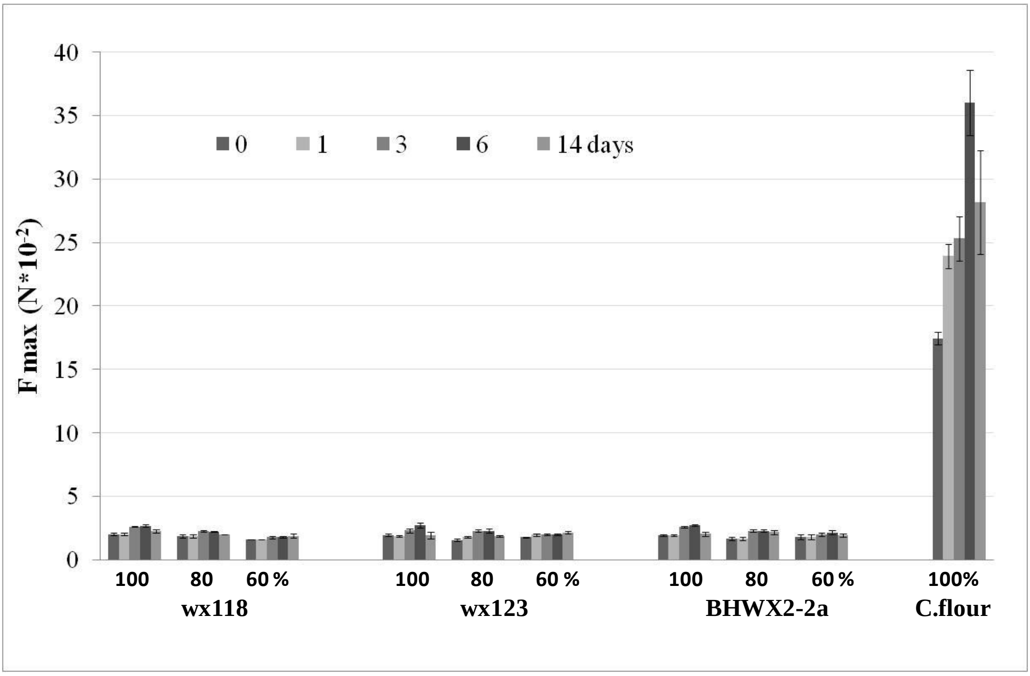
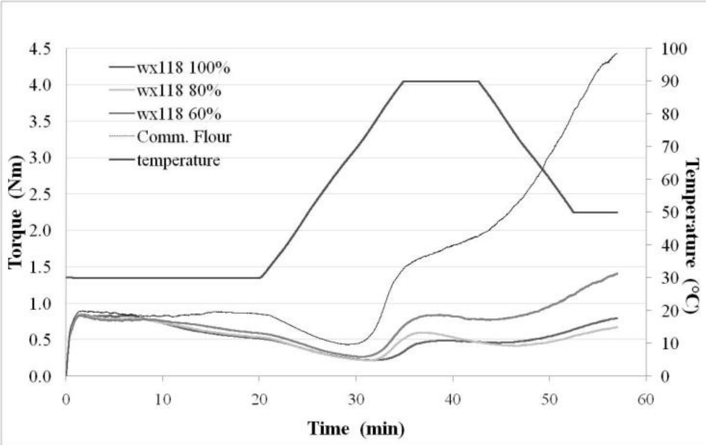


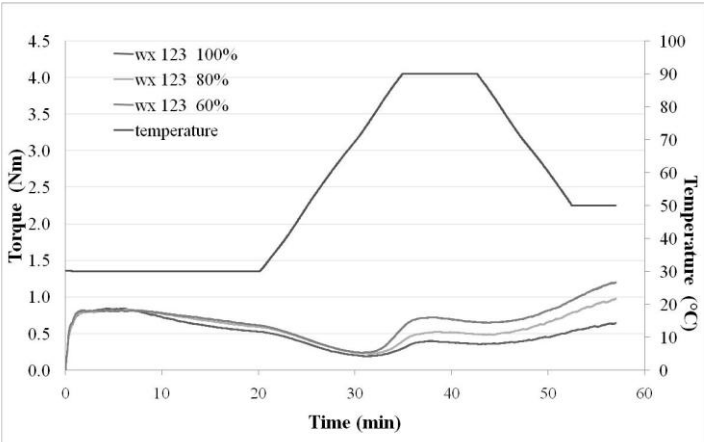
Figure 2. Firmness of RVA gels of flours from wx118, wx123, BHWX2-2a and related blends, during storage at 4 °C.

In order to determine the possible influence of the mixing conditions on the further baking process, dough was subjected to a dual mechanical shear stress and temperature constraint using the Mixolab device. Information concerning mechanical and thermal protein weakening, starch gelatinization and starch gelling can be extracted from the recorded curves (Rosell et al. 2009).

Data concerning waxy dough stability registered during the first 20 minutes of mixing at 30 °C (Figure 3) are consistent with data provided by the farinograph. In all cases considered the water amount assuring the optimal consistency (500 BU) for the farinograph test gave a consistency lower than 1.1 Torque usually proposed as optimal for the Mixolab test. During the first phase, dough stability increases and dough softening decreases for waxy blends with higher percentage of commercial flour. During the subsequent heating phase the dough consistency decreases and achieves a minimum value which is lower and is achieved at a higher temperature (75 °C) for waxy dough compared to dough from blends with commercial flour which achieve a minimum torque value at 71-73 °C. The decreasing of dough consistency during the heating phase is associated with the weakening of the protein structure due to the effects of temperature increase and applied forces during the dough mixing. The higher torque values observed during this phase in case of dough from blends can be explained in terms of increased amylose content. During the heating phase the starch granules absorb water available in their surrounding and swell, increasing the viscosity when the amylose chains, from commercial flour, leach out in aqueous inter-granular space (Rosell et al. 2009)

Figure 3 also shows that consistency achieves a maximum during the heating phase at 90 °C and that this maximum becomes significantly higher for waxy dough samples from blends with higher commercial flour content due to their higher non-waxy starch content having delayed hydration and gelatinization consequently increasing dough consistency. During the heating phase at 90 °C dough consistency starts decreasing and increases again during the subsequent cooling phase. The dough consistency increase during the cooling phase is due to the gelation process of the starch. The re-association between the starch molecules is related to the retrogradation and reordering of the starch molecules themselves. Dough consistency is higher in blends with higher commercial flour content also during this final phase mainly because of the presence of both higher amylose content and higher protein content due to the non-waxy flour. The differences in consistency observed among blends with different commercial flour content were not observed in the RVA profiles of related gels because in this latter case the high water content impeded that the different protein contents could affect the final viscosity.





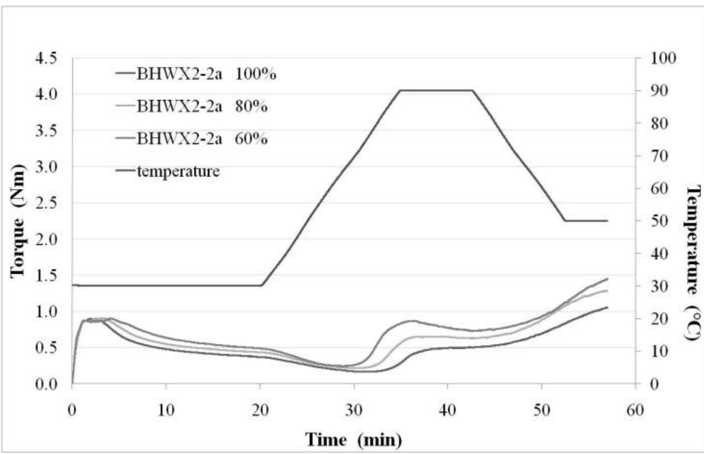


Figure 3. Mixolab profiles of dough samples from wx118, wx123, BHWX2-2a and related blends.

Dough rheological properties

The rheological analyses and the meaning of the main parameters used have already been introduced under Chapter 3. This analysis has been repeated for each of blends considered in this study and the LVE of dough samples was identified up to 0.03% strain also in case of these blends.

The values registered for the elastic modulus (G′) and the viscous modulus (G″) varied within the same ranges observed in case of dough samples from pure waxy or pure non waxy flours confirming a solid elastic-like behavior with G′ prevailing over G″ also for blends samples for all frequencies considered. Interestingly, the addition of commercial flour in waxy wheat flour did not in general cause a shift of the values of the frequency sweep curves towards the values observed for the curves corresponding to pure commercial flour, neither in case of G′, nor in case of G″. For example, in case of dough samples from wx123 and wx70 the frequency sweep curves nearest to the curve of the commercial flour were those of dough samples from pure wx123 and wx70, whereas curves corresponding to their blends with commercial flours resulted characterized by lower values both for the G′ and G″ modulus (see Figure 4). Moreover it may be worth observing that among all dough samples the dough from the blend of wx118 flour with 60% of commercial flour was the only one whose G′ and G″ values exceeded those of dough from pure commercial flour, whereas the dough from pure wx118 flour showed the lowest G′ and G″ values for all frequencies together with the dough from pure BHWX14-7 (see Figure 4). Overall, the visco-elastic behavior of dough samples from IWWLs does not seem to be markedly affected by the addition of non-waxy flour, excepting for the dough sample from wx118 flour.

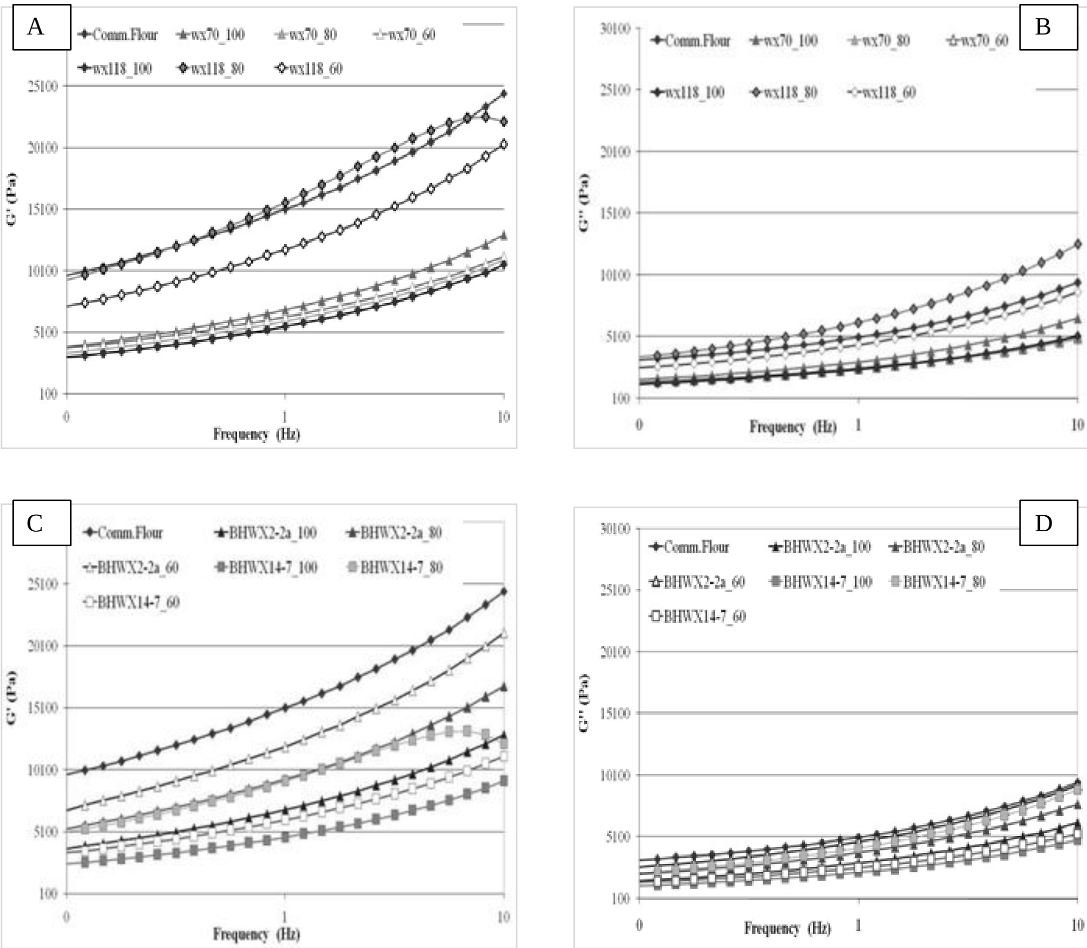


Figure 4. Frequency sweep curves (0.03 strain, 25 °C) of blends of waxy wheat flour with commercial flour. Curves are the mean of at least two replicates. A and C: storage modulus G′ (Pa). B and D: Loss modulus G″ (Pa).

Bread making properties

The characteristics of breads produced from the blends considered in this study are summarized under Table 3.

Specific volumes of breads from IWWLs are significantly higher than specific volumes of control even in case of blends with commercial flour. In case of breads from wx70 and wx118 the specific volume does not seem to be affected by the increasing percentage of commercial flour in the blends. Table 3 also indicates that breads from waxy wheats have all a comparable backing loss which is generally significantly higher than that observed in breads from pure commercial flour.

Table 3. Physic characteristics of bread samples from waxy wheat flours.

	Composition of blends	Bread				
Lines	waxy flour (g/100 g)	Volume (mL)	Weight (g)	Height (mm)	Specific Volume (mL/g)	Baking loss (%)
wx 70	100	208 fg	32.7 efg	73.2 b	6.37 fg	26.6 cd
	80	223 il	32.8 efg	81.0 fgh	6.81 hi	26.1 bcd
	60	228 l	32.8 fg	81.1 gh	6.93 i	25.3 b
wx 118	100	219 hi	33.0 g	77.7 cd	6.62 gh	26.3 bcd
	80	221 il	32.2 bcd	80.9 fgh	6.88 hi	26.9 cdef
	60	213 gh	31.6 a	78.6 de	6.72 hi	25.3 bcd
wx 123	100	226 il	33.0 g	81.1 gh	6.89 hi	28.0 g
	80	229 l	33.0 g	84.4 i	6.92 i	26.5 cd
	60	200 ef	32.6 defg	80.3 efg	6.12 ef	26.0 bc
BHWX14-7	100	185 c	32.8 fg	76.4 c	5.66 bc	27.8 fg
	80	191 cd	32.3 cd	81.1 gh	5.94 de	27.7 fg
	60	195 de	32.1 bc	82.6 hi	6.10 ef	27.0 def
BHWX2-2a	100	163 a	32.4 cde	63.6 a	5.08 a	27.7 efg
	80	191 cd	32.4 cdef	73.3 b	5.91 cde	26.7 cde
	60	185 c	32.8 efg	79.0 def	5.67 bcd	27.0 def
Comm. Flour	0	173 b	31.8 ab	71.2 b	5.41 b	23.7 a

Values followed by same letter in the same column are not significantly different ($P<0.05$).

When cells area of breads from blends with commercial flour is considered it can be observed that the addition of non-waxy flour in waxy breads did not determine an increase in the ratio between bread slice cell area and bread slice total area both in blends with 80% and 60% of waxy flour, excepting American waxy breads for which 60% blends gave a significantly higher ratio (Figure 5). This increase can be explained by observing that the density of small cells increased by about 15% in American breads from blends with 40% of commercial flour whereas this density did not change appreciably in case of IWWL breads with a the same amount of commercial flour added. The number of small cells per cm^2 ranged between 70 and 80 in all waxy wheat breads and the number of medium and large cells per cm^2 ranged instead respectively between 4 and 6 and between 1 and 2 in all waxy wheat breads and did not change appreciably in the blends with commercial flour considered.

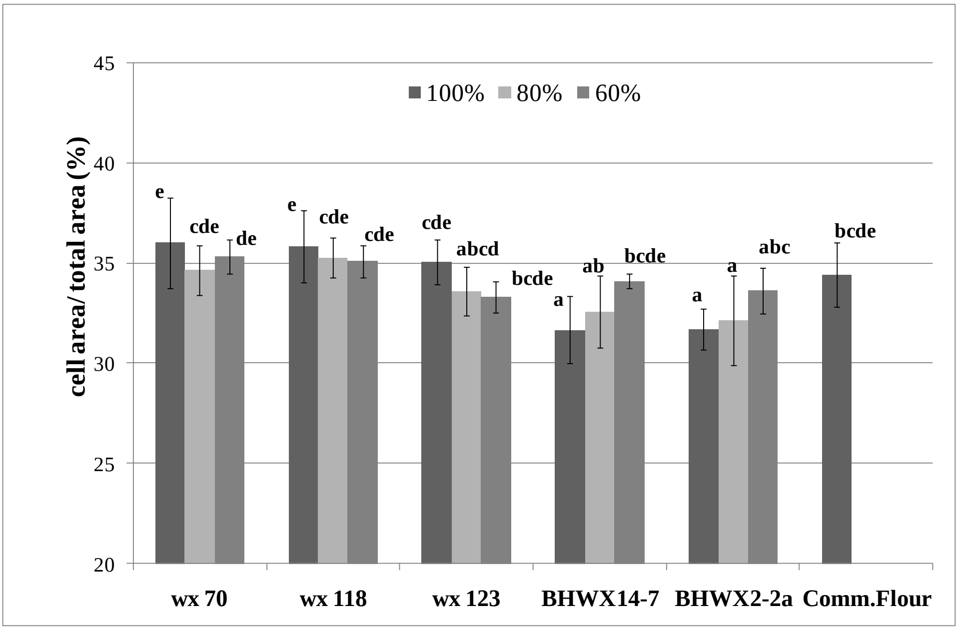


Figure 5. Cell area/total area (%) of slices of waxy breads and related blends.

Concerning bread crust color, data in Table 4 indicate that breads from pure IWWL flour have lower lightness (L), lower redness (a*) and lower yellowness (b*) with respect to commercial flour breads. This still holds for the breads from blends with IWWL flour excepting for redness (a*) which is comparable to that registered for breads from pure commercial flour.

As regards bread crumb color, the lightness (L*) is comparable for all pure waxy breadcrumbs considered and is significantly lower with respect to pure commercial flour bread lightness. In case of waxy breadcrumb from blends the lightness (L*) increases significantly with respect to that of breadcrumb from pure waxy flour but remains significantly lower than that of breadcrumb from pure commercial flour. Redness (a*) and yellowness (b*) of breadcrumb is quite comparable among all breads considered when waxy breadcrumbs from blends are excluded, with wx118 breadcrumb having a yellowness significantly lower than that of commercial flower. Redness (a*) of waxy breadcrumbs does not seem to be affected by the addition of commercial flour. Yellowness (b*) of breadcrumbs from blends with commercial flour is on average significantly lower than that of breadcrumbs from pure commercial flour only in case of blends with 60% of waxy flour.

Table 4. Color and mean area cells of Italian waxy breads and related blends.

Lines	Composition of blends waxy flour (g/100 g)	Bread crust color L*	a*	b*	Bread crumb color L*	a*	b*	Mean area small cell (mm^2)	medium cell (mm^2)	large cell (mm^2)
wx 70	100	43.84 abc	9.55 efg	24.69 a	49.57 abcd	-1.82 ab	10.39 bcde	0.06 a	1.18 c	18.28 abc
	80	43.72 abc	10.36 fgh	24.49 a	53.30 cde	-2.04 a	8.45 ab	0.17 b	1.14 bc	16.58 ab
	60	45.168 bcd	10.33 gh	25.37 ab	62.58 fg	-1.76 abc	9.53 abc	0.17 b	1.07 a	17.47 abc
wx 118	100	44.14 abc	9.37 cde	24.36 a	44.17 ab	-1.51 bc	8.59 ab	0.06 a	1.16 bc	20.28 cd
	80	44.24 abc	9.74 efg	25.43 ab	53.73 cde	-2.03 a	9.43 abc	0.18 b	1.14 bc	20.78 cd
	60	46.45 cd	10.08 efgh	28.73 cd	57.072ef	-1.78 abc	8.85 ab	0.17 b	1.13 abc	17.21 abc
wx 123	100	47.94 d	8.56 bcd	27.3 bc	49.41 abc	-1.79 ab	9.09 ab	0.06 a	1.15 bc	19.68 bcd
	80	43.09 ab	10.22 efgh	24.40 a	55.99 de	-1.99 a	11.81 e	0.17 b	1.12 ab	22.27 d
	60	46.02 cd	9.89 efgh	28.12 cd	54.40 cde	-1.82 ab	9.22 ab	0.17 b	1.12 ab	18.90 abcd
BHWX14-7	100	44.05 abc	8.42 bc	24.13 a	44.29 ab	-1.70 abc	11.71 de	0.06 a	1.14 bc	18.41 abc
	80	44.05 abc	9.40 def	25.40 ab	52.49 cde	-2.08 a	9.82 abcd	0.14 b	1.14 bc	18.73 abcd
	60	42.158 a	9.93 efgh	24.48 a	53.19 cde	-1.83 ab	8.27 a	0.14 b	1.11 ab	20.53 cd
BHWX2-2a	100	53.71 e	6.15 a	28.83 cd	43.05 a	-1.34 c	10.19 abcde	0.05 a	1.17 bc	15.98 a
	80	53.74 e	7.78 b	29.13 cd	49.84 bcd	-1.79 ab	10.01 abcde	0.17 b	1.16 bc	17.33 abc
	60	53.80 e	8.24 b	29.67 d	56.06 def	-1.72 abc	8.47 ab	0.17 b	1.15 bc	15.78 a
Comm. Flour	0	52.35 e	10.81 h	32.73 e	64.92 g	-1.54 bc	11.25 cde	0.07 a	1.13 abc	18.461 abc

Values followed by same letter in the same column are not significantly different ($P<0.05$).

In order to assess bread staling, bread crumb moisture, water activity and firmness have been analysed on day 0, 1, 3 and 7 after baking in case of breads from blends with commercial flour.

Concerning breadcrumbs moisture, on day 0 after backing IWWL breadcrumbs from blends with commercial flour have a moisture mostly comparable to that of breadcrumbs from pure IWWL flour .and higher or comparable to that registered for breads from pure commercial flour (Figure 6). Breadcrumbs from blends with 60% of IWWL flour in particular have always higher moisture with respect to pure commercial flour. On day 7 the moisture of bread from blends is generally comparable with moisture of bread from pure waxy slour and the moisture of bread from commercial flour.

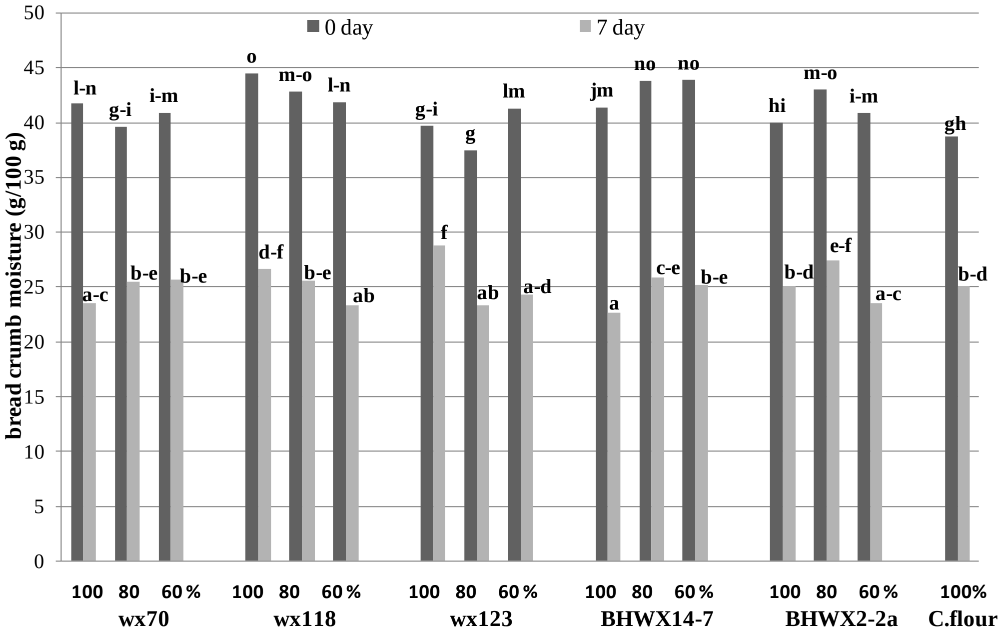


Figure 6. Moisture of breadcrumb from waxy wheat and related blends on day 0 and 7 after baking .

Figure 7 indicates that all samples have comparable crumb water activity on day 0. The value of this parameter markedly decreases on the following days for all crumbs considered, as expected. In general the addition of commercial flour does not always produce the same effects on crumb water activity during the days considered after backing. For example, the presence of commercial flour causes a significant decrease in crumbs from wx118 flour on day 7, whereas this phenomenon is not observed with wx70 series. On day 7 after backing the water activity of crumbs from waxy blends with commercial flour is always comparable to that of crumbs from pure commercial flour, excepting blends with 60% of wx118 and BHWX2-2a.

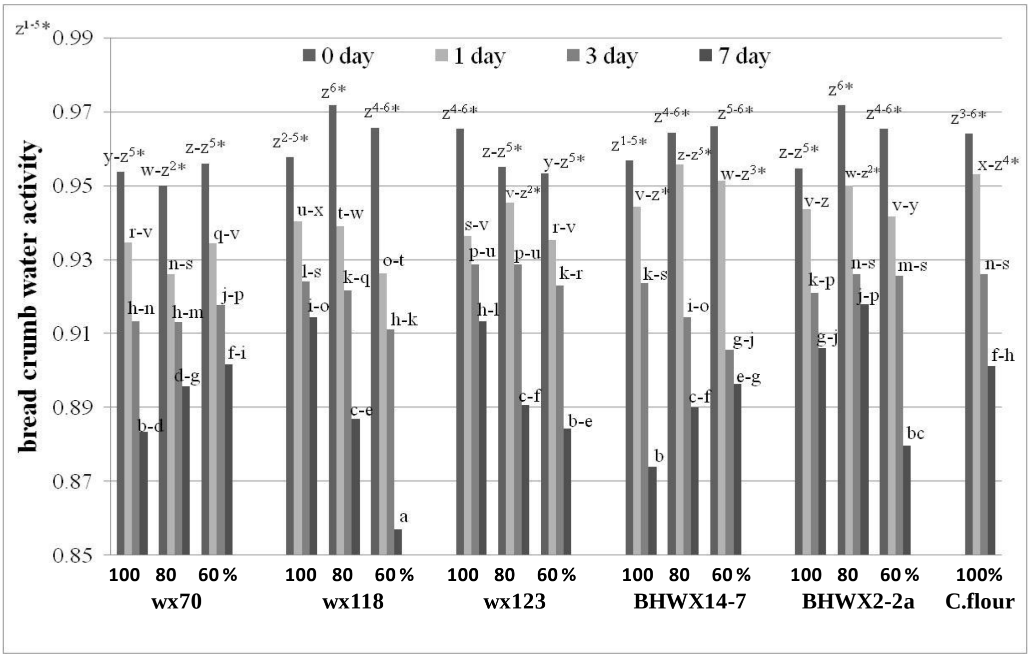


Figure 7. Water activity of breadcrumb from waxy wheats and related blends on day 0, 1, 3 and 7 after baking **.**

Concerning the firmness of breadcrumbs from blends, Figure 8 clearly shows that in case breads from IWWL the addition of commercial flour determines a very significant increase of this index on day 7 after backing for almost all breadcrumbs from 60% blends. On day 7 the breadcrumbs from pure IWWL flour that have the lowest firmness (i.e. breadcrumbs from wx123 flour) increase significantly their firmness when blends with commercial flour are considered achieving the same firmness level observed in pure commercial flour breadcrumb. Breadcrumbs from pure wx70 and pure wx118 flour maintain instead a low firmness also in case of blends with 20% of commercial flour. Finally breadcrumbs from blends with 60% of IWWL have always a firmness comparable to that of breadcrumbs from pure commercial flour on day 7. In general the firmness changes observed with the addition of commercial flour to waxy flour during the 7 days of storage seem to be correlated to combined changes in breadcrumb moisture and water activity. Indeed, it has been verified that in case of samples for which the moisture percentage decrease exceeded water activity percentage decrease after 7 days of storage, the percentage increase of firmness resulted higher, whereas the firmness percentage increase resulted lower in the opposite situation (i.e. in case of samples for which the moisture percentage decrease resulted lower than water activity percentage decrease).

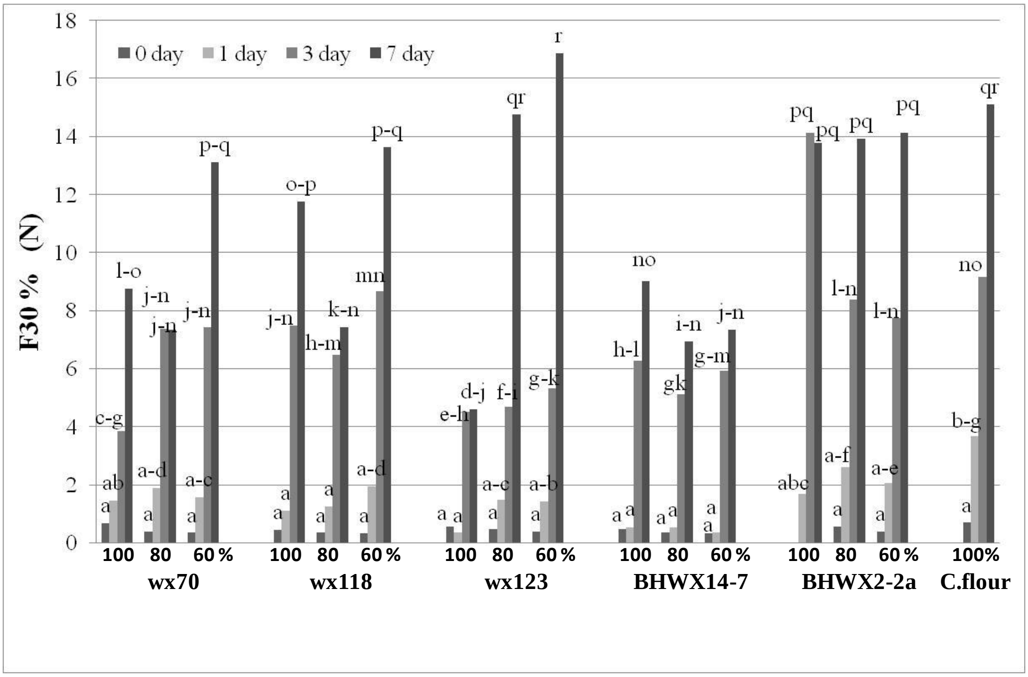


Figure 8. Firmness of waxy wheat loaves and related blends on day 0, 1, 3 and 7 after baking.

6.4 CONCLUSIONS

Waxy flours reduce bread staling but produce a dough with high adhesiveness. The employment of waxy wheat flour blends with 20 and 40% of non-waxy flour allowed reducing adhesiveness and increasing farinograph stability of dough making the dough handling easier. Starch retrogradation attitudes did not change in case of starch from IWWL flour blends with non-waxy flour also when assessed by measuring gel firmness stored at low temperatures for long time periods.

When analysed by Mixolab IWWL dough consistency resulted higher in the blends with higher non-waxy flour content.

Concerning dough rheological properties, the values registered for the elastic modulus (G′) and the viscous modulus (G″) in case of waxy dough samples with blends of non-waxy flour varied within the same ranges observed in case of dough samples from pure waxy or pure non waxy flours confirming a solid elastic-like behavior with G′ prevailing over G″ also for flour blends dough samples.

Breads obtained from IWWL flour blends with non-waxy flour resulted having specific volumes significantly higher than those of breads from American waxy flours. Crust color did not change significantly with respect to breads from pure IWWL flour excepting crust redness (a*). The lightness (L*) of breadcrumbs from blends increased significantly but remained significantly lower than that of breadcrumb from pure non-waxy flour

As regards bread cells area, the addition of commercial flour in IWWL breads did not determine on average an increase in the ratio between bread slice cell area and bread slice total area.

Finally breadcrumbs from wx70 and wx118 flour blends with 20% of non-waxy flour presented good bread-making qualities and maintained low firmness values till 7 days of storage.

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APPENDIX

AACC International Annual Meeting
October 16-19, 2011
Palm Springs, California,.USA

MACROMOLECULAR AND RHEOLOGICAL PROPERTIES OF ITALIAN WAXY WHEAT

Rosita Caramanico[1]; Patrizia Vaccino[1]; Gabriella Bottega[2]; Alberto Barbiroli[3]; Stefania Iametti[3]; Maria Ambrogina Pagani[2]

[1] CRA-SCV, Unità di ricerca per la Selezione dei Cereali e la Valorizzazione delle Varietà Vegetali, S.A. Lodigiano (LO), Italy
[2] DiSTAM, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, Milan, Italy
[3] DiSMA, Dipartimento di Scienze e Molecolari Agroalimentai, Università degli Studi di Milano, Milan, Italy

Starch retrogradation is the major cause of breadcrumb firming, and amylose is assumed to be mainly responsible for bread staling, making the food industry interested in using waxy (amylose-free) cereals and/or starches in bakery products. Waxy wheat lines have been produced in Japan and in other countries, but showed poor adaptability to the Italian agronomic conditions. A breeding program set up at CRA-SCV starting from partial-waxy cultivars identified in Italian bread wheat germplasm led to the release of about twenty waxy hexaploid wheat lines (WHW, Triticum aestivum L.). Aim of the present work is the study of the properties of these lines and of the role of the waxy trait in bread texture. Eighteen WHW were selected and characterized by chemical and physical small-scale analyses, and the pasting and rheological properties of flours were evaluated. Results were compared with those obtained from two commercial non-waxy cultivars, used as controls. Amylose content of WHW was typical of waxy lines (on average, 1.4%), with protein content ranging from 12.3% to 17.2%. In four WHW, the high protein content was associated to high gluten quality, evaluated by Gluten Index and SDS Sedimentation Volume. RVA test indicated a lower retrogradation tendency in WHW than in controls (setback values: 329 vs 931 cP). From a rheological standpoint, WHW showed high farinograph water absorption (70.3 ÷ 78.7%), but very low stability values. Baking tests indicate a good breadmaking quality of the Italian WHW, although starch-protein and protein-protein interactions in these systems deserve further investigation. This study was supported by Italian MiPAAF (CERSUOM, DM 1942/7303/08).

9th European Young Cereal Scientists and Technologists Workshop
May 19-21, 2010
Budapest, Hungary

AGRONOMICAL AND TECHNOLOGICAL PROPERTIES OF ITALIAN WAXY WHEAT

Rosita Caramanico[1], Patrizia Vaccino[1], Maria Ambrogina Pagani[2]

[1] CRA-SCV, Consiglio per la Ricerca e la sperimentazione in Agricoltura, Unità di ricerca per la Selezione dei Cereali e la Valorizzazione delle Varietà Vegetali, S.Angelo Lodigiano (LO), Italy
[2] DiSTAM, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, Milan, Italy

Food industry is increasingly interested in waxy (amylose-free) starch for its application to bakery products, because of its properties in producing a new bread texture, characterized by a soft, viscous, and glutinous crumb. Moreover, waxy starches naturally induce a delay in bread staling, extending its shelf-life. Waxy wheat lines have been already produced in Japan and other countries by using traditional hybridizations between partial waxy wheat lines lacking of one or two out of the three isoforms of the GBSS-I (Granule-Bound Starch Synthase) enzymes responsible for amylose synthesis. In order to produce waxy wheat lines in Italy A breeding programme has stated, taking advantage of autoctonous genotypes, preferred in view of their better adaptation to local environmental conditions. Aim of the present study is the agronomic and technological evaluation of a set of waxy hexaploid wheat (WHW- *Triticum aestivum* L.) obtained by screening and breeding over 300 common wheat cultivars to select *waxy* genotypes. The lines were cultivated in the growing season 2008-09 in S. Angelo Lodigiano (LO, Italy) and the main agronomic characteristics were recorded. Chemical and physical small-scale analyses were performed on kernels. Pasting properties of flours (obtained by a lab-scale milling) were analyzed by a Rapid Visco Analyzer (RVA, Foss instr.), and rheological properties were evaluated by using Farinograph and Alveograph .

CISETA 10° Congresso Italiano di Scienza e Tecnologia degli Alimenti
9-10 Maggio 2011
Milano, Italia

QUALITÀ PANIFICATORIA DI LINEE ITALIANE DI FRUMENTO TENERO WAXY

Rosita Caramanico[1], Patrizia Vaccino[1], M. Ambrogina Pagani[2]

[1]CRA – Unità di ricerca per la selezione dei cereali e la valorizzazione delle varietà vegetali, via R. Forlani 3, 26866 S. Angelo Lodigiano, Lodi

[2]Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche (DISTAM), via Celoria 2, 20133 MILANO

Abstract

L'impiego di farina di frumento tenero waxy in panificazione fino ad oggi ha dato scarsi risultati in quanto questa materia prima è di difficile lavorabilità. Recentemente si sono selezionate linee di frumento tenero italiano completamente waxy che, contrariamente al passato, mostrano migliori caratteristiche panificatorie. Nel presente lavoro, sono state messe a confronto 18 linee waxy valutandone le caratteristiche tecnologiche e l' attitudine panificatoria sia in purezza che in miscele con farina commerciale di buona qualità panificatoria. Accanto agli indici compositivi (contenuto in proteine, glutine, amilosio, amilopectina) le farine sono state analizzate con test di reologia empirica (test farinografico, alveografico) e sottoposte a test di panificazione con processo diretto (metodo AACC10-10B modificato secondo Cattaneo e Borghi,1979) ottimizzato per la fase d'impastamento, allo scopo di limitare l'adesività tipica di questa tipologia di farine. I risultati appaiono incoraggianti, anche per quanto attiene il mantenimento della sofficità della mollica per tempi prolungati.

8° CONVEGNO AISTEC
EVOLUZIONE E RILANCIO DELLA FILIERA DEI CEREALI:
11-13 maggio 2011
Aci Castello – Cannizzaro (CT), Italia

CARATTERIZZAZIONE TECNOLOGICA DI LINEE ITALIANE DI FRUMENTO TENERO WAXY

Rosita Caramanico[1], Patrizia Vaccino[1], M. Ambrogina Pagani[2]

[1]CRA-SCV, via R. Forlani 3, 26866 S. Angelo Lodigiano, Lodi;
[2]Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche (DISTAM), via Celoria 2, 20133 Milano

Abstract

L'amido, oltre a svolgere un importante ruolo nutrizionale, è uno degli elementi chiave nella determinazione della qualità dei prodotti da forno. In particolare svolge un ruolo importante nel complesso fenomeno del raffermamento, che risulta direttamente collegato alla sua retrogradazione e coinvolge in maniera differente amilosio e amilopectina, le due macromolecole di cui l'amido è composto, differenti per struttura tridimensionale e proprietà fisiche. In particolare, i dati presenti in letteratura indicano che, maggiore è la componente di amilopectina, più lenta sarà la cinetica di tale fenomeno. Da qui l'interesse verso i frumenti waxy, in cui, a causa della quasi totale assenza di amilosio, l'amilopectina risulta la componente preponderante dell'amido.

Nel presente lavoro sono state messe a confronto 18 linee di frumento tenero waxy recentemente selezionate da germoplasma italiano. Tali linee sono state valutate per le principali caratteristiche compositive (contenuto in proteine, glutine, amilosio, amilopectina) e tecnologiche (test farinografico, alveografico e viscoamilografico). Tutte le linee sono state inoltre sottoposte a test di panificazione con processo diretto (metodo AACC10-10B). In parallelo, vista l'adesività tipica di questa tipologia di farine, allo scopo di migliorarne la lavorabilità mantenendo al contempo inalterate le caratteristiche tecnologiche e di shelf-life, le linee migliori sono state analizzate in miscela, a varie percentuali, con farina commerciale di buona qualità panificatoria.

I risultati dalle analisi appaiono incoraggianti. Dal punto di vista dei parametri tecnologici, le linee sembrano avere le caratteristiche dei frumenti panificabili (FP, secondo la classificazione ISQ), mentre il test di panificazione ha evidenziato parametri tipici di classi superiori.

TECNICA MOLITORIA (2011) FEBBRAIO:101-110

QUALITATIVE CHARACTERIZATION AND BREADMAKING APTITUDE OF WAXY WHEAT LINES

Rosita Caramanico[1], Patrizia Vaccino[1], M. Ambrogina Pagani[2]

[1]CRA-SCV, via R. Forlani 3, 26866 S. Angelo Lodigiano, Lodi;
[2]Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche (DISTAM), via Celoria 2, 20133 Milano

Abstract

The increasing interest in *waxy* wheat starches by the food industry is due to their minor susceptibility towards retrogradation caused by the absence of amylose. Such property suggests that *waxy* wheat starches/cereals may be used in baking products as substitutes of the chemical additives commonly used for shelf-life extension. In the present study, partial-*waxy* and *waxy* bread wheat lines have been compared, evaluating technological quality and breadmaking aptitude in relation to amylose content decrease. Starch pasting properties of the lines were also assessed by the use of the Rapid Visco Analyser (RVA).

ANAL BIOANAL CHEM (2011) 399:1965–1973
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MINIMISATION OF INSTRUMENTAL NOISE IN THE ACQUISITION OF FT-NIR SPECTRA OF BREAD WHEAT USING EXPERIMENTAL DESIGN AND SIGNAL PROCESSING TECHNIQUES

Giorgia Foca[a], Nicoletta Sinelli[b], Manuela Mariotti[b], Mara Lucisano[b], Rosita Caramanico[c], Alessandro Ulrici[a]

[a] Dipartimento di Scienze Agrarie e degli Alimenti, Università di Modena e Reggio Emilia, Padiglione Besta, Via Amendola 2, 42100 Reggio Emilia, Italy
[b] Department of Food Science and Microbiology (DiSTAM), University of Milan, via G. Celoria 2, 20133 Milan, Italy
[c] CRA — Unità di ricerca per la selezione dei cereali e la valorizzazione delle varietà vegetali, Via Forlani 3, 26866, S. Angelo Lodigiano (Lodi), Italy

Abstract

Spectral resolution (R) and number of repeatedscans (S) have a significant effect on the S/N ratio of Fourier transform-near infrared (FT-NIR) spectra, but the optimalvalues of these two parameters have to be determined empirically for a specific problem, considering separately both the nature of the analysed matrix and the specific instrumental setup. To achieve this aim, the instrumental noise of replicated FT-NIR spectra of wheat samples was modelled as a function of R and S by means of the Doehlert design. The noise amounts in correspondence to different experimental conditions were estimated by analysing the variance signals derived from replicate measurements with two different signal processing tools, Savitzky–Golay (SG) filtering and fast wavelet transform (FWT), in order to separate the "pure" instrumental noise from other variability sources, which are essentially connected to sample inhomo inhomogeneity. Results confirmed that R and S values leading to minimum instrumental noise can vary considerably depending on the type of analysed food matrix and on the different instrumental setups, and helped in the selection of the optimal measuring conditions for the subsequent acquisition of a wide spectral dataset.

CHEMOMETRICS AND INTELLIGENT LABORATORY SYSTEMS 99 (2009) 91–100

DIFFERENT FEATURE SELECTION STRATEGIES IN THE WAVELET DOMAIN APPLIED TO NIR-BASED QUALITY CLASSIFICATION MODELS OF BREAD WHEAT FLOURS

Giorgia Foca[a], Marina Cocchi[b], Mario Li Vigni[b], Rosita Caramanico[c], Maria Corbellini[c], Alessandro Ulrici[a]

[a] Dipartimento di Scienze Agrarie e degli Alimenti, Università di Modena e Reggio Emilia, Padiglione Besta, Via Amendola 2, 42100 Reggio Emilia, Italy
[b] Dipartimento di Chimica, Università di Modena e Reggio Emilia, Via Campi 183, 41100, Modena, Italy
[c] CRA — Unità di ricerca per la selezione dei cereali e la valorizzazione delle varietà vegetali, Via Forlani 3, 26866, S. Angelo Lodigiano (Lodi), Italy

Abstract:

The Synthetic Quality Index method (Indice Sintetico di Qualità, ISQ) is used in the Italian cereal trade context for the classification of bread wheat in different quality categories, and consists in the assignation by an expert assessor of each wheat sample to the most fitting class, on the basis of parameters reflecting chemical and rheological properties of the flour. The high uncertainty of this procedure has been recently proved by some of us using a panel test, which confirmed a quite large degree of subjectivity in the assignation of samples to the quality classes. However, the results obtained with the panel test allowed to identify samples whose class assignation is sufficiently univocal, to be used for the development of automated classification methods based on NIR spectra. In the present work, multivariate classification models have been calculated using the WPTER algorithm, which aims at selecting - among the wavelet coefficients derived by application of the Wavelet Packet Transform to the analysed NIR spectra - only those features leading to the best possible discrimination among the considered classes. In particular, WPTER has been used following three different strategies to choose the optimal conditions for the development of SIMCA class models. Due to the restricted number of objects, the statistical validity of the models has been evaluated using a newly developed algorithm, which performs a double cross-validation of the SIMCA models, and by comparison with the results obtained by permutation tests.

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THE DEBRANNING OF COMMON WHEAT ON INDUSTRIAL SCALE: STUDY OF GRAIN, BY-PRODUCTS, AND FLOUR CHARACTERISTICS

Gabriella Bottega[1] - Rosita Caramanico[2] - Manuela Mariotti[1] Alessandra Marti[1] - M. Ambrogina Pagani[1*]

[1]Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche (DISTAM) - Via Celoria 2 -20133 Milano - Italia
[2]CRA-SCV, Unità di Ricerca per la selezione dei cereali e la valorizzazione delle varietà vegetali -
Via R. Forlani 3 - 26866 S. Angelo Lodigiano - LO - Italia

ABSTRACT

Thanks to the encouraging results obtained with the debranning of common wheat (pilot-plant machine), this work is aimed to verify the possibility of applying this process on industrial scale. The technological conditions were able to assure debranning level (DL) lower than 10%, allowing solely the elimination of bran layers. Pearled kernels and the corresponding flours were evaluated by means of different approaches. A general decrease in ash content was observed: the higher the DL the lower the ash content. The abrasive action did not have a significant effect on protein quality of flour, as indicated by the farinographic stability and the alveographic force (W). The general characteristics of flour were similar in comparison with those obtained using a conventional milling. Finally, regarding the microbial contamination, debranning process was significantly effective in reducing the microbial contamination of flours.

JOURNAL OF FOOD ENGINEERING 94 (2009) 75–82

THE DEBRANNING OF COMMON WHEAT (*TRITICUM AESTIVUM* L.) WITH INNOVATIVE ABRASIVE ROLLS

Gabriella Bottega[a], Rosita Caramanico [b], Mara Lucisano[a], Manuela Mariotti[a], Laura Franzetti[a], M. Ambrogina Pagani[a]

a Department of Food Science and Microbiology (DiSTAM), University of Milan, via G. Celoria 2, 20133 Milan, Italy
b CRA-SCV, via Forlani 3, 26866 S. Angelo Lodigiano (LO), Italy

Abstract:

The physical and chemical modifications associated with the debranning of common wheat (*Triticum aestivum* L.) and the improvement of the hygienic characteristics of the kernels were examined. A pilot-plant debranning machine was equipped with rolls lined with synthetic diamond powder, an innovative abrasive material. The technological parameters taken into account were: the particle size of the abrasive elements, the processing time, the number of debranning passages and the hydration conditions of the kernels before treatment. The abrasive effects were observed by Scanning Electron Microscopy and quantified by evaluating the debranning level and several chemical and physical indices of kernels and waste. A preliminary hydration passage was determinant for reducing kernel breakage during debranning. Best results in terms of homogeneous removal of bran layers were obtained with the addition of 3% water and keeping the hydrated mass mixing for 5 min. Abrasive surfaces with fine particle size (<1000 mesh) gave a quite uniform debranning process, without deep grooves in the endosperm region. A final brushing of the debranned kernels assured a more accurate removal of bran layers. Debranning levels lower than 8–10% guaranteed low starch losses in the waste and, at the same time, noticeably reduced the microbial contamination of the kernels.

TEXTURAL CHANGES OF GLUTEN-FREE PASTAS DURING COOKING AND COOKING QUALITY EVALUATION

Stefania Iametti[c] , Francesco Bonomi[c] , Maria Ambrogina Pagani[a] , Alessandra Marti[a], Rosita Caramanico[b],

[a] Department of Food Science and Microbiology (DiSTAM), University of Milan, via G. Celoria 2, 20133 Milan, Italy
[b] CRA-SCV, via Forlani 3, 26866 S. Angelo Lodigiano (LO), Italy
[c]DiSMA, Dipartimento di Scienze e Molecolari Agroalimentai, Università degli Studi di Milano, Milan, Italy

Abstract:

Pasta products made from composite flours containing neither durum nor soft wheat are highly demanded by people suffering from celiac disease. The textural quality of cooked pasta is one of the main criteria that determine consumer acceptance. The aim of this study was to evaluate textural changes of four gluten-free (GF) pastas made from either rice (100%) or rice and amaranth (75:25%) under different heat treatments and semolina pasta (control) during cooking and compare their cooking quality. The firmness and cooking quality evaluations were ndertaken according to the AACC methods. Firmness changes of pasta during cooking were measured with a texture analyzer. The cooking quality was evaluated by weight increase, solid loss into the cooking water, and texture analysis. Pasta firmness decreased with cooking time and the rate of reduction varied with the pasta sample. The optimum cooking times for GF pastas were 1–3 min higher than that of the control pasta. The percentage of weight increase during cooking was higher for the control pasta (102.7) than for pastas 1, 2, and 3 (around 70%), and the lower percentage was for pasta 4 (57.4). Solid loss (%) among the GF pastas were comparable ($p < 0.05$), but at least 5.6 times that of control pasta. All GF pastas optimally cooked showed firmness values similar to those of control, but pasta 3 presented the lower firmness value compared with the other GF pastas. The GF pastas evaluated in this study were of lower quality than control, but GF pastas could be rather acceptable if compared with other commercial GF pastas.

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NEW APPROACHES FOR SEMOLINA CHARACTERIZATION

Alessandra Marti[1], Gabriella Bottega[2], Rosita Caramanico[1]; Maria Ambrogina Pagani[2]

[1] CRA-SCV, Unità di ricerca per la Selezione dei Cereali e la Valorizzazione delle Varietà Vegetali, S.A. Lodigiano (LO), Italy
[2] DiSTAM, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, Milan, Italy

Abstract:

Semolina from durum wheat is considered the most suitable raw material for pasta-making. Protein quantity and quality are important factors affecting pasta properties, while the role of starch has received less attention. In this study two semolina samples (organic and conventional), different in protein and starch characteristics, were analyzed using both conventional and new approaches. Starch organization was studied by using DSC, MVA test, and X-ray diffraction. Rheological properties of dough during heating and cooling were investigated using Mixolab ® and Farinograph ®. Despite a lower protein and gluten content, the dough from organic semolina was stronger than conventional semolina. The organic semolina was characterized by a higher total starch content, with a higher pasting viscosity, larger gelatinization temperature range, and a higher Δ H value. Exposure of semolina to iodine vapour exhibited differences in the organization of the starch granules. Both the new approaches used to investigate dough behaviour under heating and cooling showed similar trends. More differences between the semolina samples appeared during the heating and cooling steps: the organic semolina showed a higher consistency than conventional semolina. These preliminary results suggest that there are differences in the starch properties between semolina that require further studies.

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LA DECORTICAZIONE? ANCHE PER IL FRUMENTO TENERO

Gabriella Bottega[2], Alessandra Marti[1], Rosita Caramanico[1]; Maria Ambrogina Pagani[2]

[1] CRA-SCV, Unità di ricerca per la Selezione dei Cereali e la Valorizzazione delle Varietà Vegetali, S.A. Lodigiano (LO), Italy

[2] DiSTAM, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, Milan, Italy

Abstract:

La decorticazione, convenzionalmente utilizzata nella filiera dei cereali a cariosside vestita, può essere considerata un’operazione innovativa quando applicata al frumento, cereale a cariosside “nuda”. In particolare, a fronte dei risultati positivi ottenuti nel caso del frumento duro, si è voluto studiare il possibile impiego della decorticazione nella filiera del frumento tenero, valutando gli effetti sulle granelle e sugli scarti di decorticazione, nonché sulle farine ottenute. In merito alla decorticazione su impianto pilota, è stato calcolato il livello di decorticazione (LD, ovvero la percentuale di materiale abraso) e sono stati valutati numerosi indici chimici e fisici. Le condizioni ideali di decorticazione, capaci di garantire LD pari o inferiori al 10%, sono state identificate in una preliminare fase di umidificazione della granella, un continuo rimescolamento del materiale, tempi ridotti di permanenza nel decorticatore, ripetizione del trattamento e utilizzo di mole rivestite di materiale abrasivo di fine granulometria. L’asportazione di una parte dei tegumenti ha permesso di abbreviare sensibilmente i tempi di condizionamento senza compromettere le rese di macinazione. I parametri compositivi delle farine non sono risultati compromessi, quando paragonati con quelli delle farine tradizionali. A seguito di queste valutazioni, il passaggio di decorticazione è stato inserito nel diagramma industriale di macinazione, con risultati che hanno confermato i trend positivi osservati su scala pilota.

INTERNATIONAL WHEAT QUALITY CONFERENCE : WHEAT SCIENCE : CHALLENGES & OPPORTUNITIES
2 JUNE 2009
SASKATOON, SASKATCHEWAN, CANADA

CHARACTERIZATION OF A RICE-BASED PASTA: COMPARISON WITH CONVENTIONAL SEMOLINA PASTA

Alessandra Marti[1], Rosita Caramanico[1]; Gabriella Bottega[2], Maria Ambrogina Pagani[2]

[1] CRA-SCV, Unità di ricerca per la Selezione dei Cereali e la Valorizzazione delle Varietà Vegetali, S.A. Lodigiano (LO), Italy
[2] DiSTAM, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, Milan, Italy

Abstract:

Good quality gluten-free products continue to be in demand among the celiac community. In this study we characterized the quality characteristics of gluten-free pasta made using milled and brown rice flour. Two processes were applied to produce pasta from these flours, with similar moisture contents of 40% in dough. In one case, the rice flour was processed in a conventional plant (pilot-scale) for semolina pasta where the dough temperature inside the continuous extruder was maintained at 55°C. In the second process, two extrusion steps were used: rice dough was first heated and extruded at 115° C for about 2 minutes; then the pre-treated dough was extruded again in the continuous extruder at 55°C. All samples were then dried using a low-temperature drying cycle (50 °C for 14 hours). The characteristics of rice pasta were compared with those of semolina pasta obtained by using the conventional continuous extrusion and dried using the same cycle. The weight-increase of cooked pasta, the solids loss into the cooking water and the color of the pasta were evaluated. Starch modifications induced by the pasta-making process were analyzed by using a micro-viscoamylograph test. As expected, the brown rice flour and pasta were darker compared to either white rice flour or semolina, as evidenced by a higher L, a, b values. The pasta making process did not affect the color of the samples. Following cooking, semolina pasta had the highest weight gain. White rice flour pasta made using non-conventional process had a higher weight gain than pasta from either of the brown rice pasta samples. As expected, semolina pasta had the lowest solids loss following cooking; but white rice pasta made using the non-conventional process also had similar low weight loss. Flours from rice were characterized by a lower peak viscosity compared to semolina. Starch gelatinization temperature decreased in the pasta compared to the respective flours. Furthermore, the pasting profiles were significantly influenced by the pasta making procedure. These results suggest that while good quality pasta can be made using rice flour, the structure of the pasta, as influenced by starch gelatinization, is different likely resulting in differences in weight gain and cooking losses. Further studies are underway to better understand the structure that governs a good quality rice-based pasta.

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THE QUALITY OF BREADWHEAT CULTIVARS IN 2004/2008

Rosita Caramanico[1*] - Leonardo Ormoli[1] - Renato Canestrale[2] Maria Corbellini[1]

[1]CRA-SCV, Consiglio per la Ricerca e la Sperimentazione in Agricoltura - Unità di ricerca per la Selezione dei Cereali e la Valorizzazione delle Varietà Vegetali - Via Forlani 3 - S. Angelo Lodigiano 26866 - LO - Italia
[2]CRPV, Centro Ricerche Produzioni Vegetali - Filiera Grandi Colture - Via Tebano 45 - 48018 Faenza - RA - Italia

Abstract

This paper presents the results of the qualitative analyses performed on 45 cultivars of bread wheat that were grown for at least two years during the official trials of the period 2004/2008. The park variety of bread wheat allows farmers to make targeted choices as a function of the local edaphic and climatic characteristics. The survival of bread wheat cultivation in suitable areas of northern and central Italy depends on the ability to innovate, such as the release of genotypes characterized by high nitrogen use efficiency and disease resistance.