UNIVERSITÀ DEL PIEMONTE ORIENTALE

DEPARTMENT FOR SUSTAINABLE DEVELOPMENT AND ECOLOGICAL TRANSITION

MASTER'S DEGREE PROGRAMME IN FOOD, HEALTH AND ENVIRONMENT

From Technological Quality to Food Safety: Comprehensive Assessment of Technological Traits and Deoxynivalenol Contamination in Bread Wheat Varieties Evaluated in the National Variety Network

Firmato digitalmente da Giampiero Vale
Data: 11.07.2026 21:59:09 CEST

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12.07.2026 17:23:42 GMT+02:00

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Academic Year 2025/2026

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Abstract

Bread wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide and represents a fundamental raw material for the baking industry. Its commercial value depends on both technological quality, which determines processing performance and end-use suitability, and sanitary quality, particularly with respect to contamination by deoxynivalenol (DON), one of the most prevalent *Fusarium* mycotoxins affecting wheat. Therefore, the combined evaluation of technological traits and food safety is essential for identifying high-quality wheat varieties suitable for industrial processing and safe for human consumption.

The aim of this study was to evaluate the technological and sanitary quality of bread wheat varieties included in the Italian National Variety Testing Network during the 2024–2025 growing season. Approximately 800 grain samples belonging to 49 commercial bread wheat varieties cultivated across 19 experimental locations were analysed. The technological assessment included moisture content, test weight, thousand-kernel weight, protein content, SDS sedimentation volume, and Hagberg Falling Number, while DON contamination was determined using a competitive ELISA assay. Statistical analyses were performed using the R programming language.

The results revealed considerable variability among varieties and growing locations, confirming the combined influence of genotype and environmental conditions on wheat quality. Higher-quality technological classes generally exhibited higher protein content, SDS sedimentation volume, and Falling Number values, reflecting stronger gluten and improved breadmaking potential. From a sanitary perspective, DON contamination was generally low during the 2024–2025 growing season. Most samples (69.2%) contained less than 500 μg/kg DON, while only a limited proportion exceeded the European Union maximum permitted level for unprocessed soft wheat, indicating an overall favourable sanitary status despite localized cases of higher contamination.

Overall, this study provides a comprehensive evaluation of both technological performance and food safety in bread wheat cultivated under Italian conditions. The findings contribute useful information for wheat breeding programmes, varietal selection, and the cereal industry by supporting the identification of varieties combining desirable technological characteristics with satisfactory sanitary quality.

Keywords: *Triticum aestivum* L.; bread wheat; technological quality; deoxynivalenol (DON); food safety; SDS sedimentation; Hagberg Falling Number; protein content; National Variety Testing Network.

1.Introduction

1.1 Wheat

Wheat is one of the most important cereal crops worldwide owing to its high productivity, ease of storage, and remarkable adaptability to diverse environmental conditions. Since its domestication, humans have continuously selected and improved desirable agronomic and quality traits, leading to the development of the modern wheat species cultivated today [1,2]. Wheat was first domesticated in the Fertile Crescent approximately 10,000 years ago and subsequently spread across the globe through early agricultural societies, which adapted cultivated forms to a wide range of environments [3].

Today, wheat is grown in temperate, Mediterranean, and subtropical regions of both hemispheres. Its broad geographical distribution is largely attributable to its extensive genetic diversity, which has enabled the development of more than 25,000 varieties of *Triticum aestivum* L. adapted to different climatic conditions [4].

According to sowing time, wheat is generally classified into winter and spring types. Winter wheat is sown in autumn and requires a period of vernalization during the vegetative stage, typically at temperatures between 0 and 5 °C. Approximately 80% of global wheat production is represented by winter wheat. In contrast, spring wheat does not require vernalization and is sown in spring, particularly in regions characterized by mild winters or shorter growing seasons [5].

The two most economically important cultivated wheat species are tetraploid durum wheat (*Triticum turgidum subsp. durum* Desf.) and hexaploid bread wheat (*Triticum aestivum* L.*),* which differ in genomic composition, grain characteristics, and end-use applications [6]. The domestication process resulted in important morphological modifications, including the transformation of brittle rachises into non-brittle forms and the conversion of hulled grains into free-threshing grains, thereby facilitating harvesting and cultivation practices [7].

These domestication-related changes significantly improved crop productivity and contributed to the economic importance of wheat. In addition to being a major source of carbohydrates, primarily in the form of starch, wheat also represents an important source of dietary protein for human nutrition. The principal storage proteins of the wheat endosperm, collectively known as prolamins, are classified into gliadins and glutenins according to their polymerization properties. Together, these proteins form the gluten complex, which is largely responsible for the rheological properties of wheat dough. In particular, dough elasticity and extensibility are determined by the relative proportions of glutenins and gliadins [8].

A major advantage of wheat is its versatility in the production of a wide range of food products, including bread, pasta, cakes, biscuits, and other baked goods [9]. Consequently, the cereal industry requires wheat varieties with specific quality attributes tailored to different end uses. Wheat is commonly classified and traded according to several quality parameters, including test weight, grain protein content, and rheological characteristics [10]. These market requirements have driven the

development of increasingly targeted breeding strategies aimed at improving both the quantity and quality of grain proteins [11,12].

1.2 Origin and Evolution

The history of wheat dates back approximately 10,000 years and represents a fundamental chapter in the development of agriculture [2]. Wheat is believed to have been first domesticated in the Fertile Crescent, a region of the Middle East extending from present-day Jordan, Palestine, and Lebanon to Syria, Turkey, Iraq, and Iran [8] **(Figure 1).**

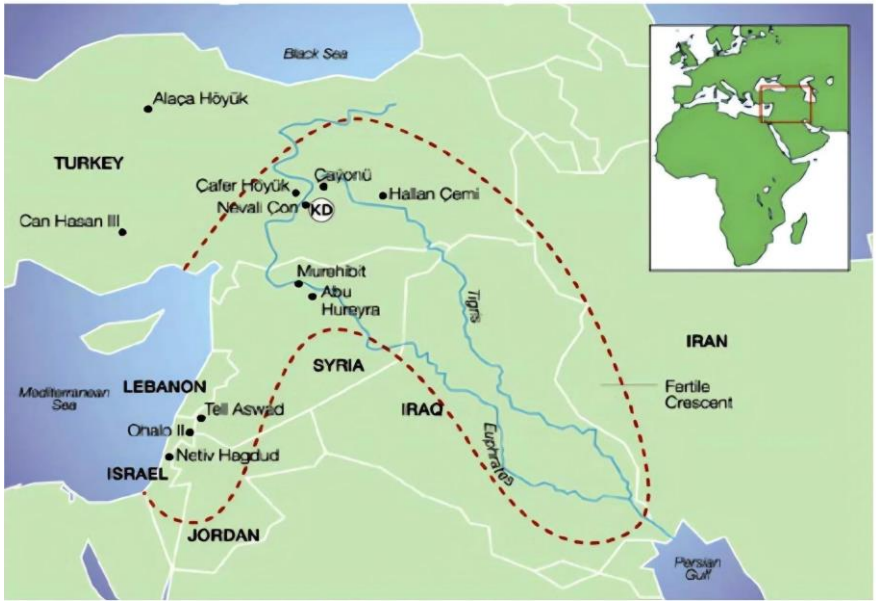

Figure 1: The Fertile Crescent and the main archaeological sites (KD: Karacadag Mountains in Turkey). Adapted from Salamini et al. 2002 [8].

Around 12,000 years ago, the Fertile Crescent experienced the transition from hunter-gatherer societies to early agricultural systems, marking the beginning of the Neolithic Revolution [8]. Even today, this region remains an important center of genetic diversity, harboring ancestral forms of cultivated wheat as well as numerous wild relatives [15].

Within the genus *Triticum* (family *Poaceae*), four principal groups of cultivated species are generally recognized [16], each characterized by a multiple of a basic chromosome number of seven:

• *Triticum monococcum* — einkorn wheat (2n = 2x = 14; genome AA; diploid)

• *Triticum turgidum* — emmer wheat, durum wheat, and related forms (2n = 4x = 28; genome AABB; tetraploid)

• *Triticum timopheevii* — Timopheev's wheat (2n = 4x = 28; genome AAGG; tetraploid)

• *Triticum aestivum* — common or bread wheat and spelt (2n = 6x = 42; genome AABBDD; hexaploid)

Although *T. timopheevii* has remained restricted to a limited Transcaucasian region and has had relatively minor agricultural importance, the other groups have played a major role in cereal production, particularly durum wheat (*Triticum turgidum* subsp. *durum*) and bread wheat (*Triticum aestivum* L.).

The genus *Triticum* represents one of the most remarkable examples of evolution through polyploidization, a widespread evolutionary mechanism that has contributed significantly to the diversification and adaptation of many plant species [6,9] **(Figures 2).**

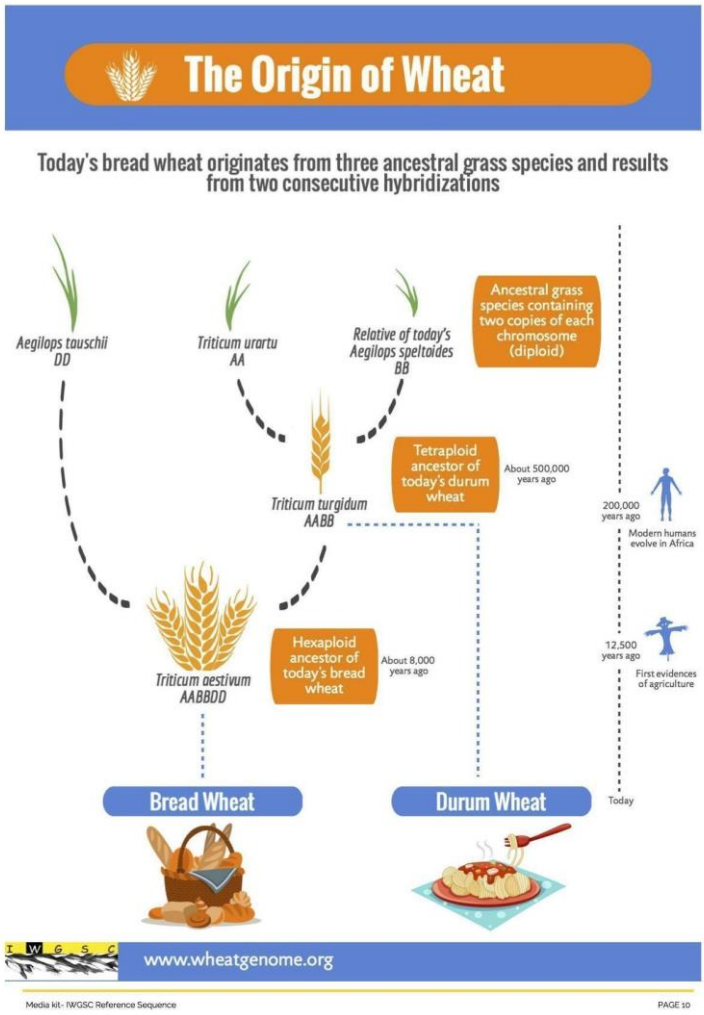

Figure 2: The origin of soft wheat and durum wheat. Adapted from the International Wheat Genome Sequencing Consortium (IWGSC), www.wheatgenome.org.

Among the cultivated species of the genus Triticum, durum wheat (*Triticum turgidum subsp. durum*) and bread wheat (*Triticum aestivum* L.) are of particular agronomic and economic importance. Their origin is the result of successive hybridization and polyploidization events that occurred during wheat evolution.

The first polyploidization event involved hybridization between two diploid progenitor species: *Triticum urartu* (AA; 2n = 2x = 14), which contributed the A genome, and *Aegilops speltoides*,

considered the donor of the B genome. This event gave rise to the wild allotetraploid emmer wheat, *Triticum dicoccoides* (AABB; 2n = 4x = 28), which was subsequently domesticated into cultivated emmer wheat (*T. dicoccum*) [21,22].

The combination of genetic material from two distinct ancestral species conferred increased vigour, broader environmental adaptability, and improved agronomic performance compared with the diploid progenitors [23]. A second polyploidization event occurred through hybridization between cultivated emmer wheat (*T. dicoccum*) and *Aegilops tauschii* (DD; 2n= 2x = 14), a wild diploid species native to the region surrounding the Caspian Sea. This hybridization generated the allohexaploid ancestor of modern bread wheat (AABBDD; 2n = 6x = 42), which was subsequently domesticated into *Triticum aestivum* L. [9,21].

Among cultivated wheat species, *Triticum aestivum* L. (bread wheat) is the most economically important, comprising more than 20,000 cultivars adapted to a wide range of environments worldwide [19]. Due to its large size and complex hexaploid structure, the sequencing of the bread wheat genome represented a major challenge for many years. A significant milestone was achieved by the International Wheat Genome Sequencing Consortium (IWGSC), which successfully assembled and annotated all 21 chromosomes of the wheat genome. This effort led to the identification of 107,891 genes and more than four million molecular markers, including numerous regulatory elements involved in gene expression. Despite its complexity, coding and regulatory regions account for only a small fraction of the genome, representing approximately 2% of its total size [25].

Further advances were achieved by Ramírez-González et al. [26], who developed a comprehensive gene expression atlas of bread wheat. This resource provides detailed information on gene activity across different tissues and developmental stages, contributing to a better understanding of gene function and regulation throughout the wheat life cycle. The atlas represents a valuable tool for identifying genes associated with agronomic traits, stress responses, and grain quality, thereby supporting the development of improved wheat varieties through modern breeding programs.

1.3 Wheat Life Cycle

The life cycle of wheat can be divided into two major developmental phases and several distinct phenological stages **(Figure 3).** Phenology is the discipline that studies the different phases of a plant's life cycle in order to understand its developmental timing and how it is influenced by both internal (endogenous) and external (environmental) factors. These stages describe the progression of the plant from emergence to maturity and are essential for interpreting growth dynamics, optimizing agronomic practices, and evaluating crop performance in relation to climatic conditions.

The vegetative phase includes germination, tillering, and stem elongation, whereas the reproductive phase comprises heading, flowering, and maturation.

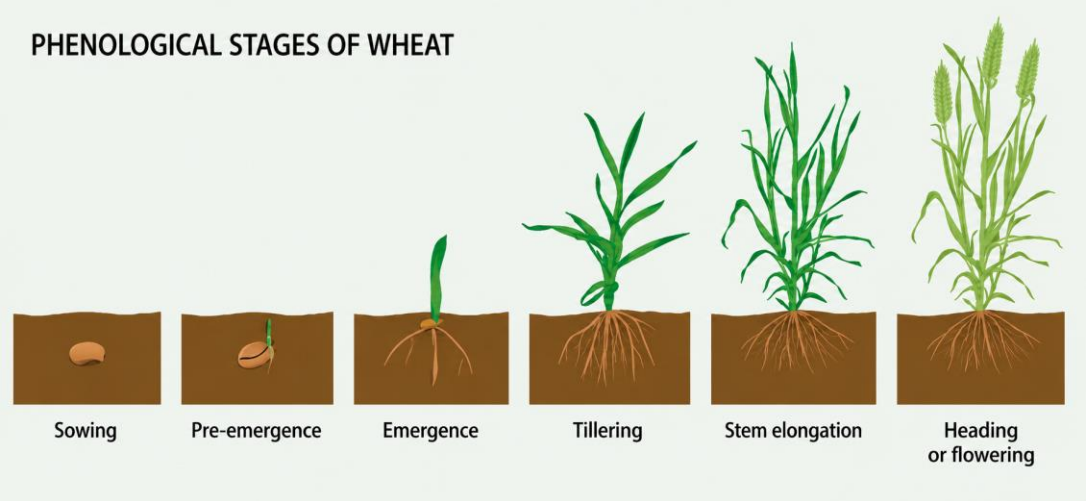

Figure 3: Phenological stages of wheat (www.gowanitalia.it).

Germination and Emergence

This phase begins when the seed absorbs water under suitable temperature and oxygen conditions. The kernel swells, metabolic activity is activated, and the seed coat ruptures, allowing the primary root and shoot to emerge. Germination includes the pre-emergence and emergence stages shown in Figure 3. During this process, the embryonic roots develop and establish the initial root system, enabling the young seedling to absorb water and nutrients from the soil.

Tillering

During this stage, the secondary root system develops and tillers (side shoots) are formed. These shoots may later develop into productive stems bearing ears. The plant remains close to the ground, increasing its resistance to low winter temperatures and adverse environmental conditions. For this reason, tillering is often referred to as the "creeping stage" of wheat development.

Stem Elongation

As temperatures increase, rapid stem growth occurs. Water and nutrient uptake reach their maximum levels, resulting in substantial dry matter accumulation. During this phase, the developing spike rises within the stem and undergoes intensive growth. Stem elongation is therefore a critical stage that strongly influences the final yield potential of the crop.

Heading and Flowering

The spike emerges from the flag leaf sheath during heading, and flowering (anthesis) generally occurs a few days later. This stage is particularly important because environmental conditions during flowering strongly influence pollination, grain set, and final yield. Adequate temperature and moisture conditions during this period are essential for successful grain development.

Maturation

Following fertilization, the grain develops through successive stages of filling and ripening until harvest maturity is reached. During this period, carbohydrates, proteins, and other reserve

substances accumulate in the kernel, contributing to grain weight and quality. This phase can be divided into four sub-stages:

- **Milk Stage**

The plant remains green, and the kernels contain milky liquid rich in nutrients. Grain filling is actively progressing, and the accumulation of reserve substances has begun.

- **Dough Stage**

The kernels become firmer and acquire a dough-like consistency while the plant gradually turns yellow. The grain continues to increase in dry weight as starch deposition progresses.

- **Physiological Maturity**

Dry matter accumulation is complete, and grain moisture decreases to approximately 25–30%. At this stage, the kernel has reached its maximum dry weight and further growth ceases.

- **Full Maturity**

Grain moisture reaches approximately 12–13%, allowing safe harvesting and storage. The plant is completely dry, and the crop is ready for harvest.

Soft wheat grows best in medium-textured and clay soils, while it performs poorly in sandy, nutrient-poor, or acidic soils. Yield per hectare can vary considerably depending on several factors, the most important being seasonal climatic conditions and the crop rotation adopted. In northern Italy, average yields typically reach 6–7 tonnes per hectare.

In agricultural practice, wheat varieties are classified into two groups:

- Winter wheats, which have a longer growth cycle and must be sown before winter (from mid-October to mid-November).
- Spring wheats, which do not require vernalization and can therefore be sown as late as March.

Fertilization plays a major role in determining productivity, as it influences the availability of essential macro- and micronutrients for the plant. Effective fertilization requires evaluating several factors, including:

- the crop's ability to exploit soil resources,
- soil fertility,
- and rainfall patterns.

Nitrogen is the primary nutrient supplied during the plant's life cycle and has a direct impact on the protein content of the kernels.

Phosphorus enhances disease resistance by promoting root development, while potassium improves resistance to lodging and is commonly applied in the form of potassium chloride.

Regarding water supply, soft wheat is typically grown in temperate climates and generally does not require irrigation, except in particularly dry or drought-prone years [27].

1.4 Structure of the wheat kernel

The wheat kernel, scientifically referred to as a caryopsis, is the fruit of the wheat plant and typically measures between 4 and 8 mm in length, depending on genotype and environmental conditions. Like other cereal grains, the mature kernel encloses a single seed that remains fused to the fruit wall at maturity. Kernel colour is largely determined by pigments and phenolic compounds located in the outer layers of the grain **(Figure 4).**

The kernel is composed of three principal anatomical fractions: the bran, the endosperm, and the germ. Each fraction differs in structure, chemical composition, and physiological function, contributing uniquely to both grain development and nutritional value.

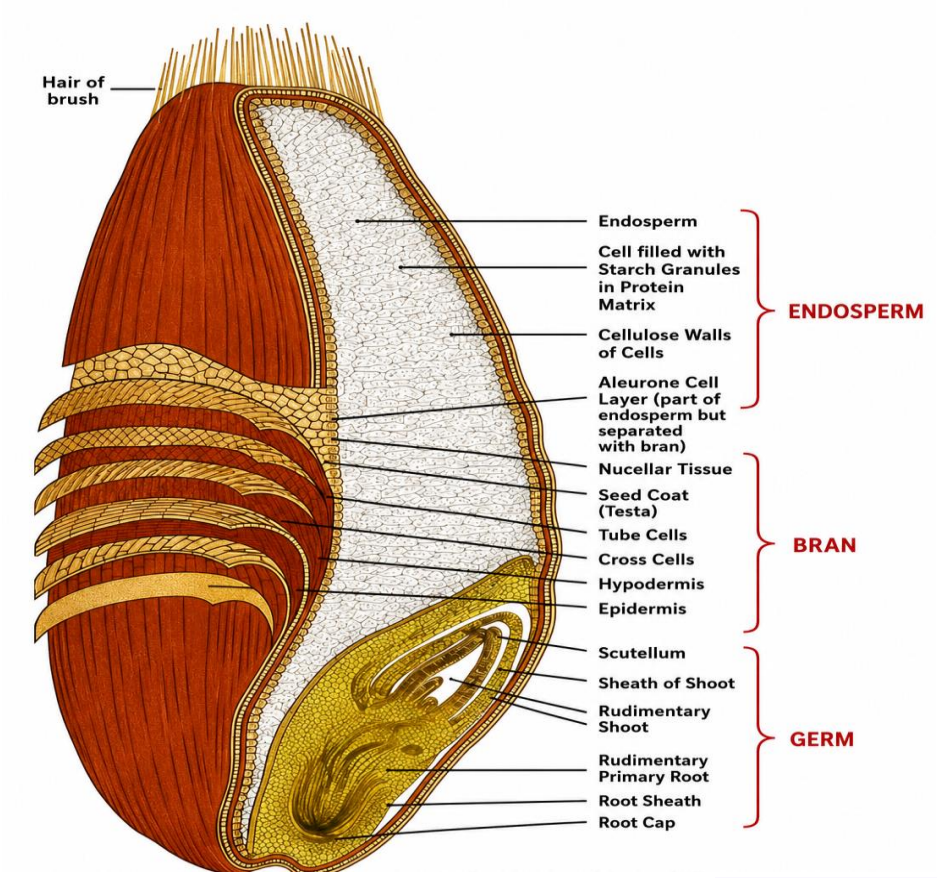

Figure 4: Longitudinal section of wheat kernel. From Cornell H.J. and Hoveling A.W. 1988. In 'Wheat, chemistry and utilization' Taylor & Francis Group, ISBN: 978-1-56676-348-6.

1.4.1 Bran

The bran constitutes the outer protective layers of the wheat kernel and includes the pericarp, seed coat, nucellar tissues, and the aleurone layer **(Figure 5).** The pericarp is composed of the epidermis, hypodermis, and several layers of thin-walled cells, forming a protective barrier

approximately 50 µm thick. Beneath the pericarp lies the seed coat and nucellar epidermis, followed by the aleurone layer, which surrounds the endosperm.

Beyond its protective role, the bran is characterised by a high concentration of dietary fibre, minerals, vitamins, and various phytochemicals. As a result, this fraction contributes substantially to the nutritional quality of whole-grain products. During milling, a large proportion of the bran is removed to produce refined flour, leading to a reduction in fiber and micronutrient content.

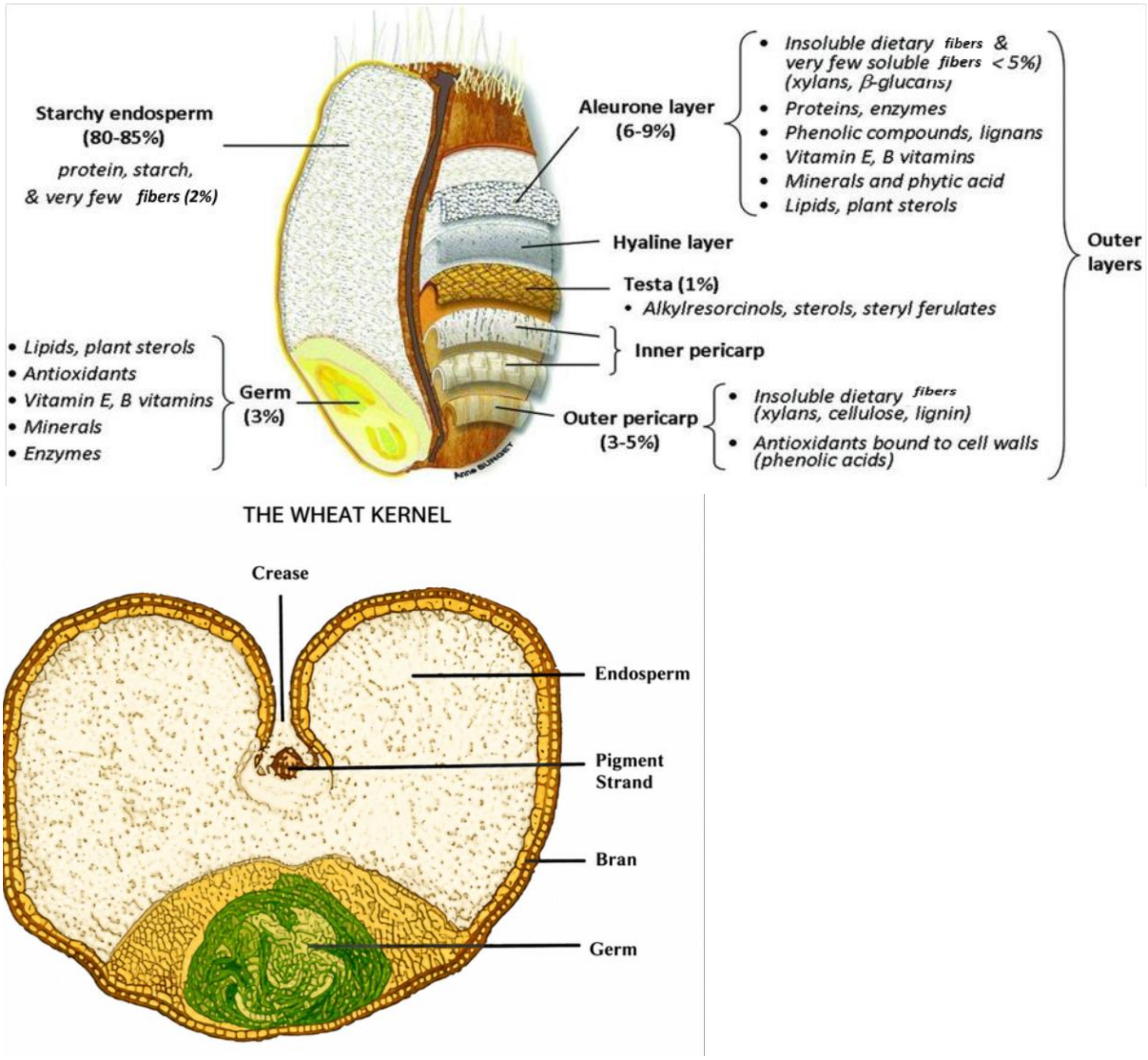

Figure 5: Longitudinal (upper figure) and transversal (lower figure) sections of the wheat kernel. From: Cornell H.J. and Hoveling A.W. 1988. In 'Wheat, chemistry and utilization' Taylor & Francis Group, ISBN: 978-1-56676-348-6.

1.4.2 Endosperm

The endosperm represents the largest proportion of the wheat kernel and serves as the primary storage tissue for nutrients required during germination. It is the principal source of white flour and consists predominantly of starch granules embedded within a protein matrix.

The protein fraction comprises albumins, globulins, gliadins, and glutenins. Gliadins and glutenins interact during dough formation to produce gluten, a viscoelastic network that plays a crucial role in the processing and baking performance of wheat flour.

Starch occurs as two distinct populations of granules. Large lenticular A-type granules range from approximately 10 to 50 μm in diameter, whereas smaller spherical B-type granules generally measure between 2 and 5 μm. Together, these granules constitute the major carbohydrate reserve of the grain and provide the energy required for seed germination and early plant growth.

Although the endosperm contains lower concentrations of micronutrients than the outer layers of the kernel, it remains the most important fraction for flour production due to its abundance of starch and storage proteins.

1.4.3 Germ

The germ, or embryo, represents the living portion of the wheat kernel and is responsible for the development of a new plant following germination. It consists of the embryonic axis, including the plumule and radicle, together with the scutellum, which functions as a storage, digestive, and absorptive organ.

The plumule develops into the aerial portion of the plant and is enclosed by the coleoptile, a protective sheath that safeguards emerging tissues during germination. The scutellum plays a central role in nutrient mobilisation by absorbing and transferring compounds from the endosperm to the developing seedling.

Although it accounts for only a small proportion of the kernel mass, the germ is characterised by a high concentration of lipids, vitamins, minerals, and bioactive compounds. In particular, it represents one of the richest natural sources of vitamin E, mainly in the form of tocopherols and tocotrienols. Owing to its nutritional value, wheat germ is frequently utilised as a dietary supplement and functional food ingredient [27].

1.4.4 Nutrient distribution within the kernel

Nutrients are distributed unevenly throughout the wheat kernel. The endosperm is rich in starch and storage proteins, whereas the bran and aleurone layers contain most of the dietary fibre, minerals, and phytochemicals. The germ is especially enriched in lipids, vitamin E compounds, and other bioactive constituents.

This heterogeneous distribution has important nutritional implications. Refining processes that remove the bran and germ improve flour shelf life and technological performance but simultaneously reduce the concentrations of fibre, vitamins, minerals, and other health-promoting

compounds. As a result, whole-grain products retain a broader spectrum of nutrients than refined wheat flour.

1.5 Chemical composition and nutritional value of wheat

Wheat is one of the most important cereal crops worldwide and serves as a major source of energy, protein, dietary fibre, vitamins, minerals, and numerous bioactive compounds. Its nutritional composition is influenced by genetic, environmental, and agronomic factors, as well as by milling and processing conditions.

1.5.1 Carbohydrates and starch

Carbohydrates constitute the largest component of the wheat kernel, accounting for approximately 70–75% of the grain dry weight. The majority of these carbohydrates occur in the form of starch, which serves as the principal energy reserve of the seed.

Wheat starch consists of two glucose polymers: amylose, a predominantly linear molecule, and amylopectin, a highly branched polysaccharide. The relative proportions of these components influence important functional properties such as gelatinisation, digestibility, texture, and processing behaviour.

In addition to starch, wheat contains smaller quantities of non-starch polysaccharides, including arabinoxylans, cellulose, and β-glucans. These compounds contribute to dietary fibre content and exert beneficial physiological effects.

1.5.2 Proteins

Proteins represent the second most abundant component of wheat grain, typically accounting for 8–20% of dry matter, depending on genotype and growing conditions. The protein fraction is commonly classified into albumins, globulins, gliadins, and glutenins according to their solubility characteristics [28].
Gliadins and glutenins are collectively known as gluten proteins and are responsible for the unique rheological properties of wheat dough. Gliadins contribute viscosity and extensibility, whereas glutenins provide elasticity and strength. The balance between these protein fractions determines dough quality and ultimately influences the characteristics of baked products.

Beyond their technological importance, wheat proteins contribute significantly to dietary protein intake and supply essential amino acids, although lysine is generally considered the limiting amino acid.

1.5.3 Lipids

Although lipids account for only a minor proportion of the wheat kernel, they play important roles in grain physiology, storage stability, and the technological properties of wheat-based products. Lipids are unevenly distributed throughout the kernel. The germ represents the richest lipid fraction, containing approximately 11% lipids, whereas smaller amounts are associated with the bran tissues and the starch–protein matrix of the endosperm.

The lipid fraction comprises phospholipids such as phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine, together with lysophospholipids. Sterols are also present, predominantly β-sitosterol, campesterol, and saturated C28–C29 sterols.

The fatty acid profile is characterised by a predominance of linoleic acid (C18:2), accompanied by lower proportions of oleic acid (C18:1) and palmitic acid (C16:0). These compounds contribute to membrane integrity, oxidative stability, and interactions between starch and gluten during food processing.

1.5.4 Dietary fiber

The majority of wheat dietary fibre is concentrated in the bran fraction, making whole-grain products considerably richer in fibre than refined wheat flour. Approximately 53% of bran dry matter consists of fibre, mainly cellulose and pentosans, commonly referred to as arabinoxylans.

Arabinoxylans constitute the major hemicellulose component of wheat cell walls and are particularly abundant in the bran and aleurone layers, where they contribute to both structural integrity and physiological functionality. Within the large intestine, these polysaccharides undergo microbial fermentation, leading to the production of short-chain fatty acids, especially butyrate. Such metabolites have been associated with improved intestinal health and a reduced risk of colorectal disorders.

Wheat also contains β-glucans, although at lower concentrations than cereals such as oats and barley. Typically present at levels around 0.6%, these polysaccharides are mainly localised within the aleurone and sub-aleurone layers. Despite their relatively low concentration, wheat β-glucans have attracted considerable interest because of their potential role in modulating postprandial glycaemic responses and supporting healthy lipid metabolism.

1.5.5 Vitamins and bioactive compounds

Wheat provides a range of bioactive compounds that contribute to both grain stability and human health. Among these, tocopherols and tocotrienols are particularly important due to their antioxidant properties.

The germ fraction contains the highest concentrations of α-tocopherol and β-tocopherol, whereas the bran is richer in α-tocotrienol and β-tocotrienol. Owing to their antioxidant activity, these compounds contribute to grain stability and have been associated with protective effects against oxidative stress and cardiovascular disorders.
Among the bioactive compounds present in wheat, carotenoids are of particular nutritional interest due to their antioxidant properties and their role as precursors of vitamin A. Lutein and zeaxanthin are the predominant carotenoids, while β-carotene occurs in smaller quantities. In addition to their antioxidant activity, some carotenoids serve as precursors of vitamin A. Ancient wheat species, especially einkorn (*Triticum monococcum*), exhibit substantially higher carotenoid concentrations than modern bread wheat and are therefore considered valuable genetic resources for biofortification and nutritional enhancement programmes.

1.5.6 Minerals and trace elements

Wheat contributes essential minerals to the human diet, particularly iron (Fe) and zinc (Zn), both of which are critical for immune function, enzymatic activity, and normal growth and development. Given the widespread prevalence of micronutrient deficiencies worldwide, wheat remains an important dietary source of essential minerals, particularly in populations where cereal-based foods constitute a major proportion of daily energy intake.
The distribution of minerals within the kernel is highly uneven. The embryo and aleurone layers contain the highest concentrations of iron and zinc, whereas the endosperm contains substantially lower levels. Zinc concentrations in the endosperm average approximately 15 mg/kg, highlighting the nutritional losses associated with flour refining.

Selenium represents another nutritionally important trace element supplied by wheat and wheat-based foods in many regions of the world. In several regions of the world, wheat-based foods provide a substantial proportion of total dietary selenium intake. Grain selenium concentrations vary considerably according to soil characteristics and typically range from 0.02 to 0.60 mg/kg. This trace element plays an essential role in antioxidant defence systems, immune function, and thyroid hormone metabolism [29].

1.6 Wheat grain quality

The concept of food quality is multifaceted and may assume different meanings depending on the stage of the production chain under consideration. Food quality is safeguarded through specific legislation and regulatory standards, while its characteristics can be quantified through physical, chemical, biochemical, and technological analyses. Standardization, testing procedures, and quality assessment methods are established by both national and international organizations, including UNI, CEN, ISO, ICC, AACC, and AOAC [30].

In the case of soft wheat, quality encompasses a wide range of attributes, including nutritional value, agronomic performance, kernel characteristics, safety, and suitability for industrial

processing. Consequently, wheat quality cannot be defined by a single parameter. Rather, it is a multidimensional concept that integrates several aspects of grain performance and end-use functionality. Among these, technological quality and hygienic–sanitary quality are particularly important, as they represent fundamental requirements throughout the cereal production chain.

1.7 Technological quality

Technological quality refers to the suitability of wheat and wheat-derived products for industrial processing and their ability to produce finished products with desirable characteristics. In bread wheat, technological quality is largely determined by the quantity and quality of storage proteins, particularly gluten-forming proteins, as well as by the rheological behaviour of dough during processing.

Italian legislation establishes quality requirements for wheat flours with respect to moisture content, ash content, and protein concentration **(Table 1).** These parameters are regulated by Presidential Decree (DPR) No. 187/2001, which defines the compositional criteria for the production and marketing of wheat flours. Moisture content is primarily related to flour storability and shelf life, whereas ash content reflects the degree of extraction and the amount of bran particles present in the flour.

Table 1: Regulatory limits for wheat flours (DPR 187/2001).

Flour type	Moisture %	Ash min.% (HCL)	Ash max. % (HCL)	Protein % (N x 5.7)
Type 00	14.5 (max 15.5)	-	0.55	9.0
Type 0	14.5 (max 15.5)	-	0.65	11.0
Type 1	14.5 (max 15.5)	-	0.80	12.0
Type 2	14.5 (max 15.5)	-	0.95	12.0
Wholemeal	14.5 (max 15.5)	1.30	1.70	12.0

Although these parameters provide important information regarding flour composition and regulatory compliance, they do not adequately describe dough rheology or predict flour performance during industrial processing and breadmaking operations [31].

A complete technological assessment requires chemical, physical, rheological, enzymatic, and functional tests **(Figure 6)**.

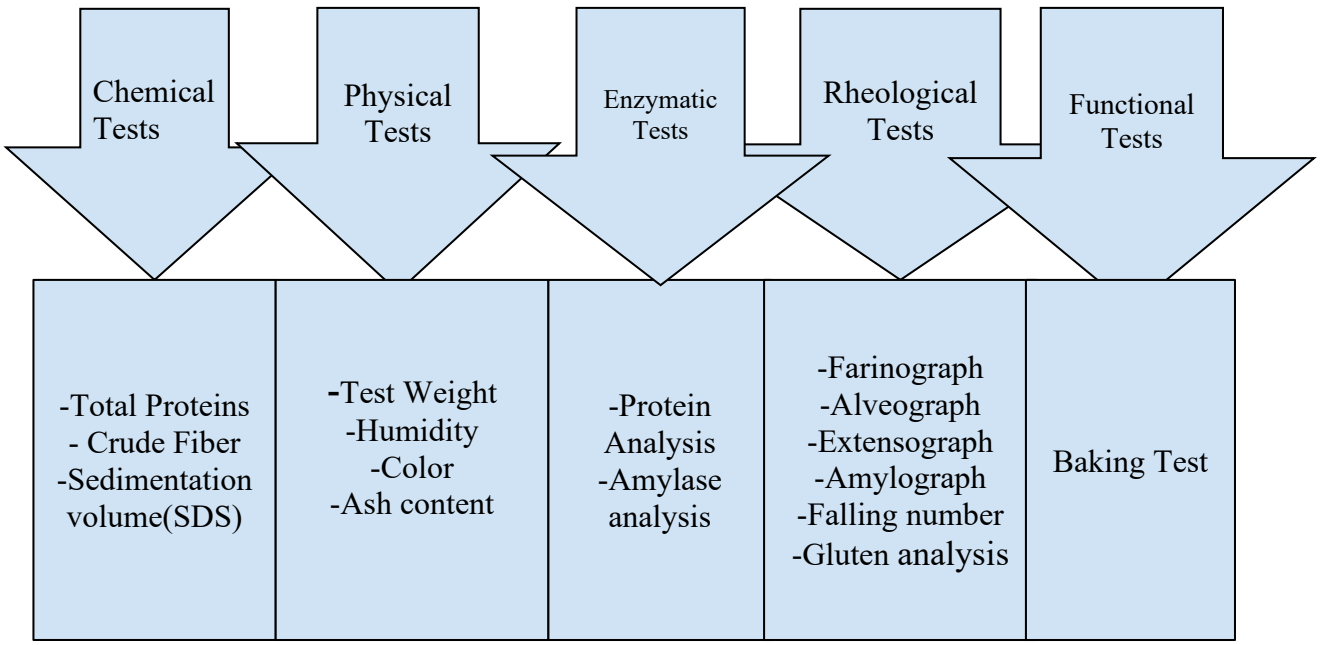

Figure 6: Main qualitative analysis carried out on soft wheat.

To overcome the limitations of compositional analyses alone, the Italian interprofessional cereal supply chain association (Assincer) introduced the Synthetic Index of Quality (ISQ), a comprehensive system designed to classify soft wheat according to its technological performance. Subsequently adopted by ITALMOPA (Italian Association of Millers and Pasta Manufacturers), the ISQ integrates compositional, rheological, and enzymatic parameters into a single quality classification framework [32].

Table 2: Classification of soft wheat according to the Synthetic Index of Quality (ISQ).				
Qualitative Parameters	Improver Wheat (FF)	Superior breadmaking wheat (FPS)	Breadmaking wheat (FP)	Biscuit wheat (FB)
Proteins (% d.m.)	12.5-14.5	11.5-12.5	10-11.5	<10
W (alveograph) J x 10^{-4}	300-340	220-300	140-220	<140
P/L (alveograph)	0.7-1.2	<0.8	<0.7	<0.5
Stability (min, farinograph)	>13	>10	>4	<4
Test weight (kg/hL)	>75	>75	>75	>75
Hagberg Falling Number (s)	>250	>220	>220	>220

The ISQ (Table 2) incorporates rheological tests (farinograph and alveograph), the determination of protein content and test weight, the evaluation of SDS-sedimentation volume, and the Hagberg Falling Number. Based on these parameters, it identifies four quality classes: **improver wheats (FF, Frumenti di Forza)**, **superior breadmaking wheats (FPS, Frumenti Panificabili Superiori)**, **breadmaking wheats (FP, Frumenti Panificabili)**, and **biscuit wheats (FB, Frumenti da Biscotto)**. All remaining wheats are classified as **wheats for other uses (FAU, Frumenti per Altri Usi)**.

This quality-based classification system offers the advantage of categorizing wheat batches according to their technological destination and has supported the establishment of differentiated market prices for the various grain types.

The protein content is a highly relevant qualitative parameter, influenced not only by genetic factors but also by environmental conditions and, in particular, by soil composition and the nutrients available within it. For this reason, the estimation of nitrogen concentration forms the basis of analytical methods used to determine protein content.

The **Chopin alveograph** is one of the most widely used instruments for assessing dough rheological properties. During the analysis, a thin sheet of dough is inflated with air until rupture, generating an alveographic curve **(Figure 7)** from which the following parameters are derived:

• P (tenacity): indicates the resistance of the dough to deformation and reflects its strength.
• L (extensibility): measures the ability of the dough to stretch before rupture.

• W (deformation energy): corresponds to the area under the curve and represents the overall strength of the dough.

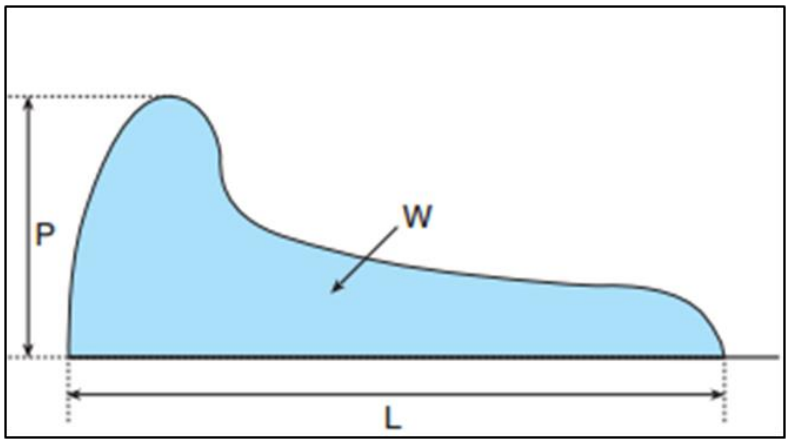

Figure 7: Schematic representation of the Chopin alveograph curve illustrating the principal dough rheological parameters: tenacity (P), extensibility (L), and deformation energy (W), used to assess dough strength and baking quality.

The **Brabender farinograph** evaluates the behaviour of dough during mixing by measuring the resistance developed against mechanical stress. The resulting farinogram provides several important quality indicators:

• Development time: the time required for the dough to reach its optimum consistency of 500 Brabender Units (BU).
• Stability: the period during which the dough maintains maximum consistency, reflecting its tolerance to mixing and fermentation.
• Degree of softening: the reduction in dough consistency after a defined mixing period, indicating dough weakening.
• Water absorption: the amount of water required to achieve a consistency of 500 BU.

Water absorption is strongly influenced by flour composition, particularly protein content and kernel hardness. Generally, an increase of 1% in protein content corresponds to an increase of approximately 1.5% in water absorption capacity.

The **test weight**, expressed in kg/hL, measures the degree of kernel filling and represents an important commercial quality indicator. Another related parameter is the **Thousand Kernel Weight (TKW)**, which is considered when estimating yield potential. TKW provides information on the milling suitability of the wheat, seed vigour, and kernel size, making it a relevant qualitative indicator [33].

The **Hagberg Falling Number** test is widely used to evaluate α-amylase activity in wheat and flour. The method provides an indirect measure of starch degradation and is therefore an important indicator of pre-harvest sprouting damage. Excessively low Falling Number values indicate elevated enzymatic activity, which may result in sticky doughs and poor bread structure,

whereas excessively high values are associated with insufficient enzymatic activity and reduced fermentation performance. Consequently, an optimal Falling Number range is essential for achieving desirable baking quality [34].

The technological quality assessment of soft wheat also requires the evaluation of additional aspects, some of which are indirectly related to its breadmaking performance. Among these, the following are noteworthy:

- **Moisture**: expresses the water content of the sample and varies according to kernel maturity; it applies to both grain and flour.
- **Sedimentation volume**: a small-scale test that allows a preliminary assessment of wheat quality, therefore its suitability for breadmaking.
- **Kernel hardness**: is another important technological trait that reflects the strength of the interaction between starch granules and storage proteins within the endosperm. These characteristics influence milling behaviour, flour particle size distribution, starch damage, and water absorption capacity. Although kernel hardness is primarily under genetic control, environmental and climatic conditions may also affect its expression. According to hardness measurements, wheat varieties are generally classified as soft, medium, or hard. Hard wheat kernels tend to fracture into larger particles during milling, whereas soft kernels produce finer flour fractions [35].

1.8 Sanitary Quality

The sanitary quality of a food product refers to its compliance with legally established food-safety standards, particularly regarding the presence of chemical contaminants, pathogenic microorganisms, and their toxic metabolites. According to the FAO, approximately 25% of global cereal production is contaminated with mycotoxins [36]. Mycotoxins are secondary metabolites produced by certain pathogenic fungi [37] that frequently infect major agricultural crops. These compounds represent one of the most significant food-safety concerns worldwide, as they can induce acute toxicity as well as carcinogenic, teratogenic, mutagenic, and immunosuppressive effects in both humans and animals [38]. The severity of these effects depends on several factors, including exposure level, target organ, age, sex, species, and physiological condition.

Among the fungal pathogens affecting soft wheat, species belonging to the genus Fusarium are of particular concern because of their ability to cause Fusarium Head Blight (FHB), one of the most destructive diseases affecting wheat worldwide. Under severe epidemic conditions, FHB may reduce grain yield by up to 70%. In wheat, the disease is characterized by bleaching and premature drying of spikelets, which appear pale in comparison with the healthy green tissues of the ear. The infection frequently results in poor grain filling, shrivelled kernels, or complete grain loss. At the plant level, Fusarium infection may impair embryo development, weaken plant vigor, induce root rot, and promote the appearance of white–pink fungal growth at the stem base, together with browning of leaf sheaths and foliage [39].

The contrast between infected and healthy tissues represents one of the most characteristic diagnostic symptoms of the disease, although this distinction becomes less evident as the crop approaches maturity. Drying of the spikelets may be either partial or complete **(Figure 8)**.

Figure 8: Fusarium-contaminated wheat in the field (L'informatore agrario).

Infected kernels frequently appear shriveled and exhibit a grayish-pink coloration; however, symptom expression may vary according to the timing of infection. Kernels infected during the later stages of grain development may retain a normal size. In durum wheat, infected kernels typically lose their characteristic translucency and become pale and opaque.

Fusarium Head Blight is caused by a complex of sixteen phylogenetically related fungal species, among which *Fusarium graminearum* and *Fusarium poae* are considered the most aggressive pathogens. Their development is strongly favored by high humidity and temperatures ranging from 18–21 °C to approximately 29.5 °C [40]. Consequently, an accurate assessment of disease severity should always take into account meteorological conditions during the growing season [41].

FHB is one of the most widespread wheat diseases globally and is responsible for substantial losses in both grain yield and quality. In addition to reducing productivity, infection raises serious food-safety concerns because many Fusarium species produce toxic secondary metabolites known as trichothecenes **(Figure 9)**. These compounds are among the principal factors affecting the sanitary quality of contaminated wheat.

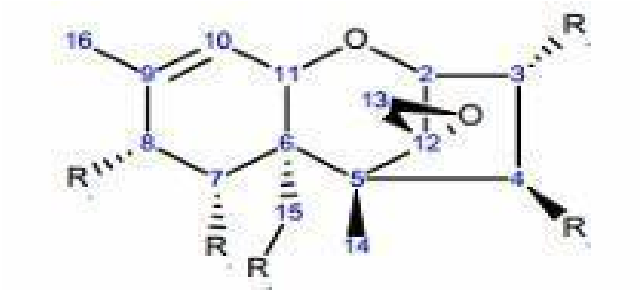

Figure 9: Chemical structure of Tricothecene.

Group A trichothecenes, including T-2 toxin, HT-2 toxin, and diacetoxyscirpenol (DAS), are generally regarded as the most toxic and have been associated with severe pathological conditions, including alimentary toxic leukopenia. Group B trichothecenes include DON, its derivatives 3-ADON and 15-ADON, and nivalenol (NIV), which can also exert severe toxic effects when present at elevated concentrations.

Trichothecenes primarily act as inhibitors of protein synthesis by targeting the peptidyl-transferase center of eukaryotic ribosomes. Their activity prevents peptide-bond formation during translation, thereby disrupting protein biosynthesis. In addition, affected cells exhibit alterations in nucleic acid metabolism, cell division, membrane integrity, and organelle functionality [44]. These mechanisms explain the broad spectrum of toxic effects associated with trichothecene exposure in both humans and animals.

Another mycotoxin of considerable concern is zearalenone (ZEN), a non-steroidal estrogenic compound produced by several Fusarium species. Owing to its structural similarity to endogenous estrogens, ZEN can interfere with reproductive and endocrine functions, leading to fertility disorders, hemorrhagic syndromes, and hepatic or renal dysfunction. The toxin is commonly detected in wheat, maize, and other cereal crops.

Given the toxicological significance of deoxynivalenol (DON), the European Union has established maximum permitted levels for its occurrence in cereals and cereal-derived products. According to current European Union legislation [65], the maximum DON concentration permitted in unprocessed common (soft) wheat intended for human consumption is 1000 µg/kg (ppb), whereas a higher limit of 1500 µg/kg is established for durum wheat **(Table 3).** These regulatory thresholds are intended to protect consumer health and ensure the safety of cereal-based foods.

The tolerable daily intake (TDI) for DON is set at 1 µg/kg body weight, based on toxicity evaluations conducted by the Food Safety Commission of Japan (FSCJ) in 2010. For nivalenol (NIV), the European Food Safety Authority (EFSA) initially proposed a TDI of 1.2 µg/kg body weight, which was subsequently revised by the FSCJ to 0.4 µg/kg body weight [44].

Table 3: Maximum permitted levels of deoxynivalenol (DON) in unprocessed wheat grains according to current European Union legislation.

Wheat type	Product category	Maximum DON level (µg/kg)
Soft wheat (*Triticum aestivum*)	Unprocessed grains intended for first-stage processing	1000
Durum wheat (*Triticum turgidum* subsp. *durum*)	Unprocessed grains intended for first-stage processing	1500

Most wheat varieties generally remain below the legal limits for DON contamination, which is essential for ensuring that flours are suitable for both feed use and direct human consumption. In Italy, however, cereal-growing areas in the northern regions show a higher risk of exceeding these legal thresholds. This is largely due to meteorological conditions that often favor the development of DON-producing fungi, particularly *Fusarium graminearum* and *Fusarium culmorum*.

Rainfall during flowering, combined with warm and humid conditions during grain filling and maturation, creates an ideal environment for fungal infection. Agronomic factors also play a crucial role, including crop rotation, management of crop residues, soil tillage practices, varietal susceptibility, and direct plant protection measures such as fungicide applications. In Italian growing environments, the most critical period for infection is the heading–flowering stage, which typically occurs in early May. During this window, it is possible to effectively reduce the risk of Fusarium infection by applying appropriate plant-protection treatments, such as foliar fungicides.

Pre-sowing agronomic practices can also significantly limit pathogen spread. For example, burying maize and sorghum residues through plowing can reduce DON contamination by more than 60% compared to direct seeding [45]. Recognizing the importance of prevention, an updated version of the guidelines for mycotoxin control in wheat and maize was published in 2021, with active contributions from CREA-CI [46].

1.8.1 Deoxynivalenol (DON)

Deoxynivalenol (DON) is the most prevalent mycotoxin detected in cereals throughout Europe and North America and is frequently found in wheat, barley, oats, and maize. It belongs to the trichothecene family of sesquiterpenoid mycotoxins **(Figure 10).**

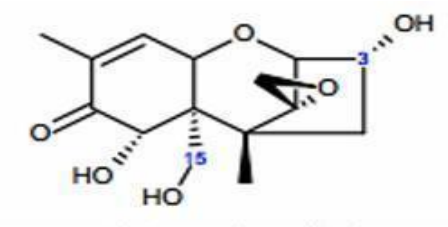

Figure 10: Chemical structure of deoxynivalenol (DON).

DON is produced as a secondary metabolite by several Fusarium species, particularly *Fusarium graminearum* and *Fusarium culmorum*. The occurrence of these pathogens is strongly influenced by climatic conditions and may vary considerably between geographical regions and growing seasons. Periods characterized by high humidity and frequent rainfall, especially during anthesis, are particularly conducive to fungal infection and subsequent mycotoxin production.

Human and animal exposure to DON may also occur through the ingestion of so-called masked mycotoxins, which are covalently or non-covalently bound to other matrix constituents. Following

ingestion, these conjugated forms may be hydrolyzed in the gastrointestinal tract, releasing free DON and increasing its bioavailability [47].

At the cellular level, DON exerts its toxic effects through binding to the A-site of the peptidyl-transferase center located on the 60S ribosomal subunit of eukaryotic cells. This interaction disrupts protein synthesis and induces ribotoxic stress, leading to the activation of mitogen-activated protein kinase (MAPK) signaling pathways. The resulting cellular response affects numerous biological processes, including cell differentiation, apoptosis, inflammatory signaling, and immune regulation [48,49].

In humans, DON exposure has been associated with several adverse health effects, including:

- Acute intoxication characterized by nausea and vomiting, which explains the common designation "vomitoxin";
- Immunomodulatory and cytotoxic effects;
- Alterations in renal function.

In livestock, consumption of DON-contaminated feed may result in reduced feed intake, impaired growth performance, and increased susceptibility to infectious diseases. Among domestic animal species, pigs are considered particularly sensitive to DON exposure.

According to the International Agency for Research on Cancer (IARC), DON and other trichothecenes are classified as Group 3 compounds, indicating that they are not currently classifiable as carcinogenic to humans. Nevertheless, epidemiological investigations conducted in the Chinese regions of Haimen and Penlai suggested a possible interaction between fumonisin B1 and other co-occurring mycotoxins, including DON, in populations exhibiting elevated rates of primary liver cancer. Although these studies did not demonstrate a direct carcinogenic effect of DON, experimental studies in animal models have indicated that DON may contribute to carcinogenic processes when present in combination with other mycotoxins [50]. Consequently, a potential co-carcinogenic role cannot be completely excluded.

In response to growing concerns regarding consumer exposure, the Directorate-General for Health and Food Safety (DG SANTE) of the European Commission has proposed lowering the maximum permitted concentrations of DON and T-2/HT-2 toxins in cereals and cereal-based products intended for human and animal consumption. Compared with the limits currently established under Regulation (EC) No. 1126/2007, the proposed reductions include:

- Unprocessed cereals: from 1250 to 1000 μg/kg;
- Durum wheat: from 1750 to 1250 μg/kg.

Additional reductions have been proposed for products intended for direct human consumption:

- Milled cereal products and pasta: from 750 to 500 μg/kg;
- Bread, pastries, and biscuits: from 500 to 400 μg/kg;
- Processed cereal-based foods and foods intended for infants and young children: from 200 to 150 μg/kg [51].

Industrial processing may contribute to the partial reduction of DON contamination, with reported decreases of up to 33% during breadmaking and related processing operations [52]. During these treatments, DON can be transformed into degradation products such as isoDON, norDON B, and norDON C, which have been reported to exhibit lower cytotoxicity than the parent compound [48].

The effectiveness of DON reduction depends largely on the processing conditions employed. Reported decreases include:

- 0–21% during cracker production;
- 4–16% during biscuit production;
- 2–5% during breadmaking.

Higher baking temperatures, longer processing times, and increased concentrations of sodium bicarbonate ($NaHCO_3$) have been shown to enhance DON degradation. Conversely, ingredients such as ammonium bicarbonate (NH_4HCO_3), yeast, vinegar, and sugar appear to have little or no effect on DON reduction [48].

These findings emphasize the importance of both preventive agricultural practices and appropriate processing technologies in limiting DON contamination and improving the sanitary quality of cereal-based products.

1.9 Varietal Trials

In Italy, a National Varietal Testing Network for the major cereal crops has been operating for more than forty years. The network plays a fundamental role in evaluating and disseminating information on the agronomic performance, technological characteristics, and sanitary quality of the most important cereal varieties cultivated throughout the country. Consequently, it represents a valuable decision-support tool for breeders, farmers, millers, processors, and other stakeholders involved in the cereal production chain.

For soft wheat, the varieties included in the annual varietal trials are selected according to specific criteria:

- The two to four most widely cultivated varieties at the national level, identified on the basis of seed certification data provided by CREA-DC (Research Centre for Plant Protection and Certification) for the previous growing season.
- Four to five varieties of particular agronomic and technological interest, in. the tester for each ISQ class.
- Newly registered varieties included in the National Register for which the breeder has requested pre-certification, as well as varieties registered in previous years that are still undergoing seed multiplication and have not yet been evaluated within the network.
- Imported varieties listed in the European Union Common Catalogue, provided that they have been certified in Italy for at least two consecutive years and have achieved an annual certified seed production of no less than 500 tonnes.

Each variety remains within the testing network for two consecutive years and is evaluated under a wide range of environmental conditions across the national territory. Field trials are conducted at approximately 20–30 locations using replicated experimental plots and standardized testing protocols, ensuring the reliability and comparability of the collected data.

Since 2020, the National Varietal Testing Network has been coordinated by CREA-CI (Research Centre for Cereal and Industrial Crops) located in Vercelli, which oversees the organization of the trials, data collection, and dissemination of results.

2. Aim of the study

The aim of this study was to evaluate the technological and sanitary quality of bread wheat (*Triticum aestivum* L.) cultivated under different environmental conditions within the Italian National Variety Testing Network during the 2024–2025 growing season.

The research was conducted within the framework of the RETICER project and involved the analysis of approximately 800 grain samples belonging to 49 commercial bread wheat varieties representing the main Italian quality classes (FF, FPS, FP, and FB).

The study aims to assess the technological quality of the varieties through small-scale analyses: protein content and kernel texture determination, using near-infrared spectroscopy (NIR), sedimentation volume (Zeleny test), and evaluation of alpha-amylase activity through the Hagberg falling number test. In addition, the sanitary quality of the samples was assessed through the detection of deoxynivalenol (DON) using ELISA-based methods.

By comparing technological and sanitary traits across varieties and locations, the study aims to better understand how both varietal characteristics and environmental factors shape wheat quality. At the same time, it supports the development of essential research skills, including laboratory organization, teamwork, data interpretation, and clear scientific communication.

Ultimately, this work contributes to improving the evaluation of soft wheat in Italy and strengthens the scientific basis of national varietal assessment.

3. Materials and Methods

The research involves the analysis of 49 modern soft-wheat varieties, covering the full spectrum of Italian quality classes — FF (Frumenti di Forza; improver wheats), FPS (Frumenti Panificabili Superiori; superior bread wheats), FP (Frumenti Panificabili; bread wheats), and FB (Frumenti da Biscotto; biscuit wheats) **(Table 4).**

Table 4: Soft wheat varieties analysed: name and year of release.

Variety	Year of release
Improver wheat (FF)	
Apulia	2022
Bologna	2002
Clarabella	2023
Giorgione	2013
LG Anouk	2023
Rebelde	2012
Superior Bread making wheat (FPS)	
Algeri	2020
Artek	2022
Brenda	2022
Eolo	2022
Garbino	2020
KWS Alpinum	2021
KWS Milanum	2021
Pasodoble	2023
RGT Impavido	2021
RGT Propulso	2022
RGT Toronto	2022
Su Vermillon	2022
Bread making wheat (FP)	
Alagir	2022
Atene	2023
Atlanta	2022
Austin	2023
Bandera	2008
Basaltic	2021
Brigitta	2022
Carlotta	2023
Clotilde	2023
Coyote	2020
Frida	2022
KWS Flexum	2021
LG Alvarez	2021

LG Arecibo	2021
Pinokio	2023
Providence	2019
RGT Annapolis	2020
RGT Bookmaker	2023
RGT Pantone	2020
RGT Scrambler	2022
RGT Parka	2022
Solehio	2008
SY Lirico	2021
SY Olen	2020
SY Passion	2020
SY Milteo	2021
Zandalee	2023
Biscuit wheat (FB)	
Ampara	2022
Concetta	2023
Modern	2013
Van Gogh	2024

The materials were cultivated during the 2024–2025 growing season in 26 locations **(Figure 11)** belonging to the Italian national varietal testing network, and representing a wide range of agro-ecological conditions across Italy.

Figure 11: Geographical distribution of the field trial locations across Italy and partner collaborating to the activities.

Unfortunately, damage caused by wildlife, particularly wild boars, continues to be a serious problem in the Lazio region. As in the previous growing season, the trial conducted in Rome was completely lost and therefore could not be included in the study. In addition, the trial conducted in Catania exhibited high variability in the data and, due to various technical issues, experienced partial to complete grain losses in some plots. Consequently, it was excluded from the analyses.

After harvest, the samples from the following locations were sent to **CREA-CI Vercelli**: Quargnento (AL), Cigliano (VC), Poirino (TO), Voghera (PV), Stezzano (BG), Piacenza, Ceregnano (RO), Lonigo (VI), Fiume Veneto (PN), Pozzuolo del Friuli (UD), Conselice (RA), Fiorenzuola d'Arda (PC), Indice (BO), Roccastrada GR), Alberese (GR), Cesa (AR), Jesi (AN), Tolentino (MC), Tarquinia (VT), Foggia, Larino (CB), Libertinia (CT). Upon arrival, they were stored, prepared, and analysed following standardized protocols.

The analytical equipment used in the study includes:

- Laboratory balances for thousand kernel weight assessment
- Grain Analysis Computer (GAC) for test weight determination
- Cyclotec™ laboratory mill for whole-grain grinding
- NIR spectrometer for protein content and kernel texture
- Hagberg Falling Number instrument
- Zeleny sedimentation apparatus
- ELISA kits for the detection of deoxynivalenol (DON)
- Standard laboratory tools for sample handling, weighing, and data recording

These materials form the basis for evaluating the technological and sanitary characteristics of the wheat varieties across different Italian environments.

3.1 Thousand Kernel Weight (TKW)

Thousand kernel weight (TKW) was determined according to the official **ISO 520** method. Two subsamples of 100 kernels were counted and weighed for each wheat line, and the average value was extrapolated to 1000 kernels and expressed in grams. TKW is an important physical quality parameter that provides information on kernel size and the degree of grain filling, reflecting both varietal characteristics and growing conditions.

3.2 Test weight

Test weight was determined using a Grain Analysis Computer (GAC) **(Figure 12)**. The instrument measures test weight through an internal volumetric system by automatically dispensing a representative grain sample into a standardized measuring chamber of known volume. Once the chamber is filled, the mass of the grain contained within that volume is recorded. Test weight is then calculated as the ratio of grain mass to the fixed volume and is expressed in kilograms per hectolitre (kg hL^{-1}). This parameter is widely used as an indicator of grain density, kernel soundness, and overall commercial quality.

Figure 12: Grain analysis computer (GAC) used for test weight determination.

3.3 Grinding

Figure 13: Cyclotec™ Foss Mill.

The grain samples were ground using a Cyclotec™ laboratory mill (FOSS, Denmark) (**Figure 13**), a high-speed centrifugal grinding system specifically designed to produce homogeneous whole-meal flour for analytical purposes. The instrument operates through a rotor–stator mechanism, in which a rapidly rotating impeller accelerates the kernels outward against a fixed abrasive ring, generating combined impact, shear, and abrasion forces that efficiently break down the starchy endosperm and peripheral tissues. The resulting particles are forced through a 1-mm stainless-steel sieve, ensuring a standardized and reproducible particle size distribution suitable for chemical and technological analyses. This grinding principle minimizes heat development, thereby preserving the integrity of thermosensitive components such as enzymes, starch granules, and protein structures. Owing to its high reproducibility and low thermal stress, the Cyclotec™ mill is widely adopted in cereal-quality laboratories and varietal evaluation programs for the preparation of samples.

3.4 NIR (Near-Infrared Spectroscopy)

Figure 14: NIR (Near-Infrared Spectroscopy) apparatus.

Near-infrared spectroscopy (NIRS) is an advanced, rapid, and non-destructive analytical technique increasingly used in wheat-quality evaluation, cereal chemistry, and plant-breeding programs due to its ability to generate reliable compositional data with minimal sample preparation. Conventional laboratory methods for determining key technological traits—such as protein content, moisture, grain hardness, and other physicochemical properties—are often time-consuming, labor-intensive, and costly, limiting their suitability for high-throughput screening in breeding pipelines [53]. NIRS overcomes these limitations by exploiting the near-infrared region of the electromagnetic spectrum (780–2500 nm), where absorption bands arise from overtone and combination vibrations of X–H functional groups, particularly C–H, N–H, and O–H bonds [54]. These molecular vibrations produce complex but highly informative spectral patterns that reflect the chemical composition and structural characteristics of the sample. Because hydrogen-containing bonds are abundant in organic materials, NIRS can be calibrated to quantitatively predict a wide range of wheat quality parameters, including protein concentration, moisture content, starch characteristics, and kernel texture [55]. Modern NIR instruments **(Figure 14)** integrate high-precision detectors and chemometric algorithms, enabling simultaneous multi-trait prediction with excellent reproducibility. As a result, NIRS has become a fundamental tool in wheat breeding, grain quality control, and industrial processing, providing rapid, cost-effective, and environmentally sustainable analysis essential for evaluating large numbers of samples.

3.5 Hagberg-Perten Falling number (HFN)

The Falling Number (FN) test is an internationally standardized method (ICC 107/1; AACC 56-81B) used to evaluate the level of α-amylase activity in wheat kernels and flour, providing a rapid indication of pre-harvest sprouting and weather damage. Exposure of mature wheat to rainfall or high humidity prior to harvest can trigger enzymatic activation and partial germination, leading to excessive α-amylase production and degradation of starch.

Figure 15: Hagberg–Perten Falling Number apparatus.

The FN apparatus **(Figure 15)** measures the viscosity of a heated flour–water slurry, which is inversely related to the enzymatic breakdown of starch. In the standard procedure, wholemeal flour is first prepared and 7.0 g of the ground sample is added to a calibrated viscometer tube containing a fixed volume of distilled water (25 mL). The mixture is vigorously shaken to obtain a uniform suspension, ensuring that all particles adhering to the tube walls are incorporated. The tube, together with its stirring/plunger rod, is then placed in a boiling water bath maintained at 100 °C. After an initial 5-second equilibration, the instrument automatically stirs the slurry to promote starch gelatinization, followed by a period during which the plunger is released and allowed to fall freely through the thickening paste. In samples with high α-amylase activity, starch granules are rapidly hydrolysed, resulting in a low-viscosity gel through which the plunger descends quickly, producing a low Falling Number value. Conversely, samples with low enzymatic activity form a more viscous gel, slowing the plunger's descent and yielding a high Falling Number. Thus, the FN value serves as a critical indicator of wheat's technological suitability for breadmaking, as excessive α-amylase activity negatively affects dough handling, loaf volume, and crumb structure [56,57].

3.6 SDS Sedimentation Volume

The SDS-sedimentation test is a widely used qualitative method for evaluating the gluten quality and breadmaking potential of wheat flours. The technique is based on the ability of gluten proteins—primarily gliadins and glutenins—to swell, aggregate, and form stable flocculates in a weakly acidic medium containing sodium dodecyl sulfate (SDS) and lactic acid, conditions that promote controlled protein hydration and aggregation [58].

In this environment, SDS disrupts non-covalent interactions within gluten proteins, while lactic acid provides the acidity required for uniform swelling, resulting in a sedimentation volume that reflects the strength and aggregation capacity of the gluten network. Higher sedimentation volumes are associated with stronger gluten and superior baking performance, making the SDS test a reliable indicator of flour quality and a standard procedure in cereal-quality laboratories [59].

Figure 16: SDS-sedimentation apparatus for simultaneous analysis of 15 flour samples.

In this study, a custom-built SDS-sedimentation apparatus capable of processing 15 samples simultaneously was used **(Figure 16)**, enabling efficient comparison across multiple wheat varieties. The test was performed on wholemeal flour prepared using the Cyclotec™ mill, with a commercial Barilla flour included as a reference standard.

Figure 17: Preparation of 2% sodium dodecyl sulfate (SDS) solution used for SDS sedimentation analysis.

Two solutions were prepared: a 2% SDS solution (20 g SDS dissolved and brought to 1 L with distilled water) **(Figure 17)** and a lactic acid solution (10 mL lactic acid diluted to 90 mL). The working solution was obtained by adding 20 mL of the lactic acid solution to the full liter of SDS solution. Each analysis was conducted in a 10 mL graduated cylinder containing 5 mL of distilled water and 0.5 g of flour, followed by a series of controlled shaking and inversion steps to ensure complete dispersion. After adding 5 mL of the working SDS–lactic acid solution, the suspension was gently mixed at defined intervals and allowed to sediment for 25 minutes.

Figure 18: SDS Sedimentation Test showing final sediment volumes of wheat flour samples.

The final sediment height was recorded **(Figure 18)** and multiplied by 10 to express the sedimentation volume relative to 100 mL, facilitating standardized comparison across samples. This method provides a robust and reproducible measure of gluten strength, supporting its long-standing use in wheat-breeding and flour-quality assessment [60].

3.7 Mycotoxin (DON) analysis in wheat flour by competitive ELISA

Figure 19: RIDASCREEN® DON Kit Components.

The RIDASCREEN® DON assay **(Figure 19)** is a competitive enzyme-linked immunosorbent assay (ELISA) designed for the quantitative determination of deoxynivalenol (DON) in wheat. DON is a trichothecene mycotoxin produced predominantly by *Fusarium graminearum* and *F. culmorum*, pathogens commonly infecting wheat under humid pre-harvest conditions. Due to its cytotoxic, immunosuppressive, and gastrointestinal effects, DON represents one of the most critical contaminants affecting the sanitary quality of wheat [61,62]. Accurate quantification is therefore essential for evaluating wheat safety and compliance with regulatory limits.

3.7.1 Preparation of wheat extracts for DON analysis

Figure 20: Weighing of wholemeal flour samples for DON extraction.

For each wheat sample, 1.00 g of wholemeal flour from each cultivar grown in each location was weighed into a clean reaction tube **(Figure 20)**. Subsequently, 5 mL of distilled water was added, and the mixture was homogenized using a vortex mixer to ensure complete wetting of the flour. To further improve extraction efficiency, all tubes were then manually mixed for 5 minutes using continuous circular motion, promoting maximal release of DON from the wheat matrix into the aqueous phase.

After homogenization, 1.50 mL were taken from each sample and transferred into 2.0 mL Eppendorf tubes. These tubes were centrifuged for 10 minutes at 5.000 rpm, allowing complete sedimentation of particulate material and producing a clear supernatant suitable for ELISA analysis.

Figure 21: Preparation of wheat extracts and dilution tubes for ELISA-based deoxynivalenol (DON) analysis.

While centrifugation was in progress, the final dilution tubes required for the ELISA were prepared and labelled. For each sample, 800 µL of MilliQ water was pipetted into a clean Eppendorf tube. After centrifugation, 200 µL of the clarified supernatant (liquid phase) was added to the dilution tube, yielding a 1:5 dilution (1 part sample extract + 4 parts water). This dilution step is essential because the RIDASCREEN® DON assay requires wheat extracts to be diluted five-fold, and therefore all final DON concentrations obtained from the calibration curve were multiplied by 25, accounting for both the extraction ratio (1 g in 5 mL) and the 1:5 dilution factor **(Figure 21).**

The diluted extracts, representing the final working solutions, if not immediately used, were stored at (2–8 °C).

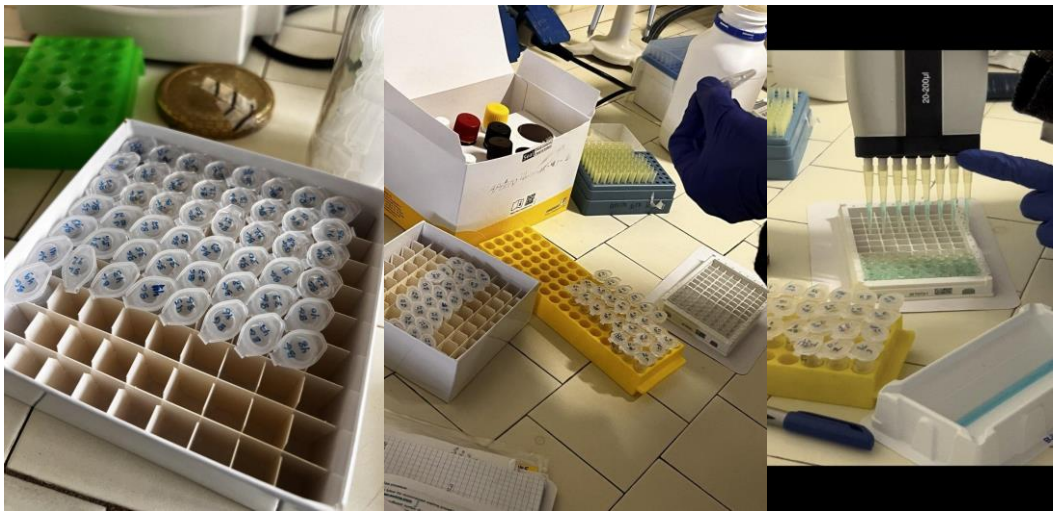

Figure 22: Preparation of standards, sample extracts, and microplate wells during ELISA-based DON determination.

The quantification of deoxynivalenol (DON) in wheat samples was performed using the RIDASCREEN® DON competitive enzyme-linked immunosorbent assay (ELISA), following the manufacturer's protocol [63] **(Figure 22)**. The assay is based on a competitive antigen–antibody reaction, in which DON present in the sample competes with a DON–enzyme conjugate for binding to specific anti-DON antibodies. The reaction takes place in microwells pre-coated with capture antibodies, which immobilize the antibody–DON complexes during the assay.

To quantify DON, the assay uses five calibration standards (0.0, 3.7, 11.1, 33.3, 100 µg/kg). These standards generate a logit/log calibration curve, where each standard's absorbance is converted into B/Bo (%), expressing the signal relative to the zero standard. In a competitive ELISA, higher DON concentrations produce lower absorbance and therefore lower B/Bo values. The curve's IC50 value (≈19.5 µg/kg) represents the concentration that reduces the signal by 50%, confirming the assay's sensitivity. A correlation coefficient of 0.999 indicates excellent curve linearity and reliable quantification across the analytical range. These parameters ensure that the DON concentrations interpolated from the curve are scientifically robust

For each analysis, the five DON calibration standards provided in the kit were pipetted into the microwells together with the samples. These standards must be included in every plate, because

the microplate reader uses them to generate a new standard curve for each run, ensuring accurate interpolation of the DON concentrations of the unknown wheat extracts. Without freshly pipetted standards, the instrument cannot calculate reliable results, as absorbance values vary slightly from plate to plate and day to day.

After placing the standards, a sufficient number of microwells were inserted into the plate holder. Into each well, 50 µL of either DON standard or diluted wheat extract was pipetted. Immediately afterward, 100 µL of the DON–enzyme conjugate (red-cap reagent) was added, followed by 50 µL of the anti-DON antibody solution (black-cap reagent). The plate was gently mixed and incubated for 30 minutes at room temperature (20–25 °C) to allow competition between free DON in the sample and the enzyme-labeled DON for antibody binding sites.

Following incubation, the wells were emptied and washed three times with 250 µL of PBS-Tween washing buffer to remove all unbound components. After washing, 100 µL of the TMB substrate solution (blue-cap reagent) was added to each well. The enzyme conjugate catalyzes the conversion of TMB into a blue-colored product, and the plate was incubated for 15 minutes at room temperature to allow color development.

The reaction was then stopped by adding 100 µL of stop solution (yellow-cap reagent, 1 N sulfuric acid), which converts the blue color to yellow. Because this is a competitive ELISA, the intensity of the yellow color is inversely proportional to the DON concentration in the sample:

- Strong yellow color → low DON concentration
- Weak yellow color → high DON concentration

Figure 23: ChroMate® microplate reader used to determine absorbance values at 450 nm during RIDASCREEN® DON ELISA analysis

Absorbance was measured at 450 nm within 10 minutes of adding the stop solution. The ChroMate reader **(Figure 23)**, uses the absorbance values of the five freshly pipetted standards to construct

a logit/log calibration curve, from which the DON concentration of each wheat sample is calculated after applying the appropriate dilution factor.

The RIDASCREEN® DON ELISA provides several analytical parameters that determine how the results must be interpreted. The method has a detection limit of 18.5 μg/kg, meaning that concentrations below this threshold cannot be reliably distinguished from background noise. Its recovery rate of 85–110% confirms that the assay accurately measures DON in wheat matrices, consistent with international validation studies [64]. These performance characteristics ensure that the method is sufficiently sensitive for evaluating DON contamination in wheat, a cereal highly susceptible to *Fusarium* infection.

Once the DON concentration of each wheat extract is calculated, interpretation must follow toxicological and regulatory criteria. According to EU legislation [65], wheat flour must not exceed 1000 μg/kg DON, making this the critical threshold for sanitary evaluation. To classify contamination levels, the following framework is usually applied **(Table 5).** This structured interpretation allows a precise evaluation of the sanitary quality of wheat samples and provides a scientifically grounded basis for distinguishing between compliant, borderline, and unsafe contamination levels. It also supports broader discussion of wheat safety, agronomic susceptibility, and the implications of DON for food quality and public health.

Table 5: Classification of deoxynivalenol (DON) contamination levels in wheat flour based on concentration ranges and regulatory relevance.

Contamination Level	DON concentration (μg/kg)	Interpretation
Safe	< 250	Indicates minimal *Fusarium* activity and no significant sanitary concern.
Moderate	250– <1000	Complies with EU regulatory limits but indicates increasing contamination; monitoring is recommended.
High	1000–1500	Exceeds the EU maximum limit for wheat flour and is considered non-compliant for human consumption.
Very High	> 1500	Indicates severe *Fusarium* infection and a substantial toxicological risk.

3.8 Statistical analysis

Data were analysed using the R programming language (R Core Team, Vienna, Austria; https://www.r-project.org/), widely used for statistical analysis and the generation of tables and graphical outputs, in combination with the free and open-source integrated development environment RStudio (Posit Software, Boston, MA, USA; https://posit.co/download/rstudio-

desktop). Descriptive statistics were computed for all measured traits. Relationships among variables were evaluated through Pearson correlation analysis, while Principal Component Analysis (PCA) was performed to identify the major sources of variation and explore associations among the technological quality parameters. Graphical representations, including boxplots, correlation matrices, and PCA biplots, were generated to facilitate data visualization and interpretation.

4. Results

The results are presented according to the main technological and sanitary quality traits evaluated in the bread wheat samples: test weight, thousand kernel weight (TKW), moisture content, protein content, Falling Number, SDS sedimentation volume (SSV), and DON contamination, as well as correlation analysis and principal component analysis (PCA). Results are discussed in relation to varietal performance, growing location, and ISQ technological quality class.

The ANOVA revealed significant differences among varieties (V) and locations (L), as well as a significant V × L interaction for all the traits under study. The following sections report the descriptive statistics for each parameter analysed.

4.1 Test Weight (TW)

The distribution of test weight among the 49 soft wheat varieties evaluated in the National Variety Trial Network is presented in **Figure 24**. Test weight showed a relatively wide range of variation, with values ranging from 63.80 kg hL^{-1} for the variety CARLOTTA grown in Alberese (GR), to 87.63 kg hL^{-1} for REBELDE cultivated in Voghera (PV).

The overall mean test weight was 79.64 kg hL^{-1}, while the median value was 79.60 kg hL^{-1}. The interquartile range extended from 77.63 kg hL^{-1} (25th percentile) to 81.70 kg hL^{-1} (75th percentile), indicating a relatively homogeneous distribution of values among the tested varieties.

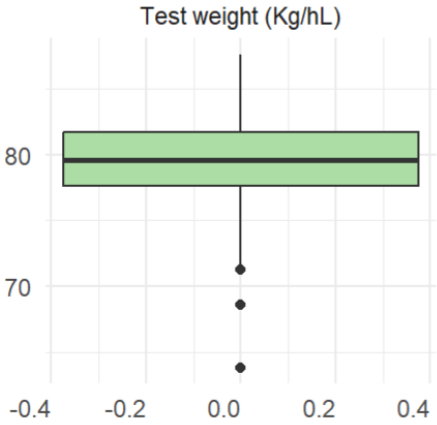

Figure 24: Box plot showing the distribution of test weight (kg hL^{-1}) among the 49 soft wheat varieties evaluated in the National Variety Trial Network.

Environmental conditions had a marked influence on this parameter. The highest average test weight was recorded in Tolentino (MC), with an average value of 83.55 kg hL^{-1}, closely followed by Conselice (RA) (83.34 kg hL^{-1}). In contrast, the lowest average test weight was observed in Poirino (TO), with 76.23 kg hL^{-1}, followed by Fiorenzuola d'Arda (PC) (76.57 kg hL^{-1}).

As far as the technological quality class (ISQ) **(Figure 25)**. is concerned, we observed that the FF class showed the highest mean value (81.40 kg hL^{-1}), whereas the FP class presented the lowest average (79.12 kg hL^{-1}). Intermediate values were observed for the FPS (79.84 kg hL^{-1}) and FB (79.30 kg hL^{-1}) classes. These differences were expected and are consistent with the existing

literature, suggesting a tendency for varieties belonging to higher quality classes to exhibit greater grain density and kernel filling.

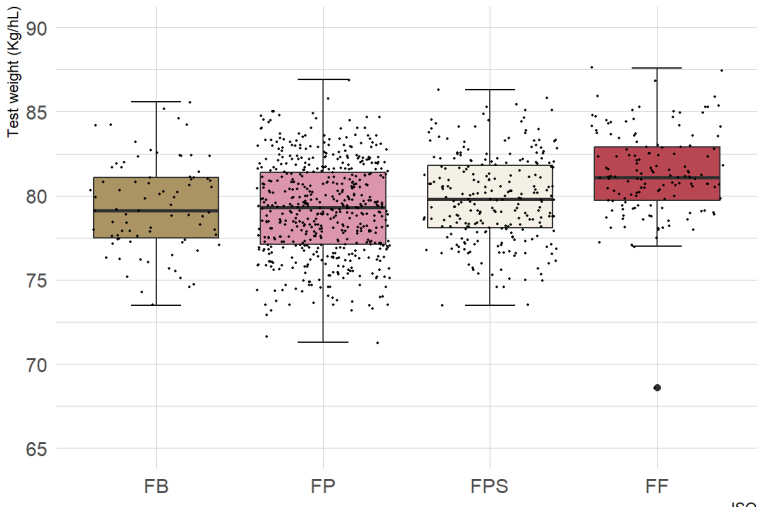

Figure 25: Box plot of test weight (kg hL^{-1}) across the ISQ classes (FB, FP, FPS and FF).

Overall, the results indicate that test weight was influenced by both varietal characteristics and environmental conditions, reflecting differences in grain development and maturation among locations.

4.2 Thousand Kernel Weight (TKW)

The boxplot in **Figure 26** represents the distribution of thousand kernel weights (TKW) among the 49 soft wheat varieties. TKW exhibited considerable variability, ranging from 22.43 g for the variety APULIA grown in Foggia to 58.95 g observed for CLOTILDE cultivated in Cesa (AR).

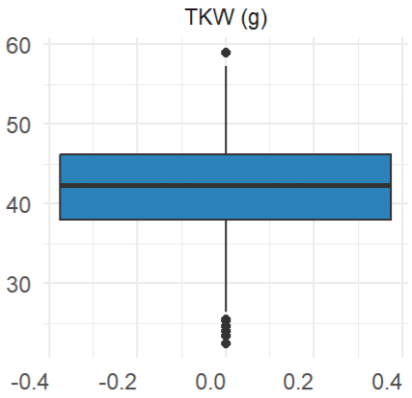

Figure 26: Box plot showing the distribution of thousand kernel weights (TKW, g) among the 49 soft wheat varieties evaluated in the National Variety Trial Network.

The overall mean TKW was 41.62 g, while the median value was 42.33 g. The interquartile range extended from 37.99 g (25th percentile) to 46.23 g (75th percentile), indicating moderate variability in kernel size and grain filling among the tested varieties.

Location had a pronounced effect on TKW. The lowest average TKW was recorded in Southern Italy, in the locations of Foggia (31.36 g), and Libertinia (32.46 g). These values reflect less favourable grain-filling conditions that occurred in the growing season during kernel development. In contrast, several northern and central Italian locations showed substantially higher kernel weights, indicating more favourable environmental conditions for grain growth.

TKW across technological quality class (ISQ) is represented in **Figure 27**. For this parameter, wheats for biscuit (FB) showed the highest average value (43.67 g), and a gradual decrease was observed from FP (41.98 g) to FPS (41.96 g) and FF (38.50 g).

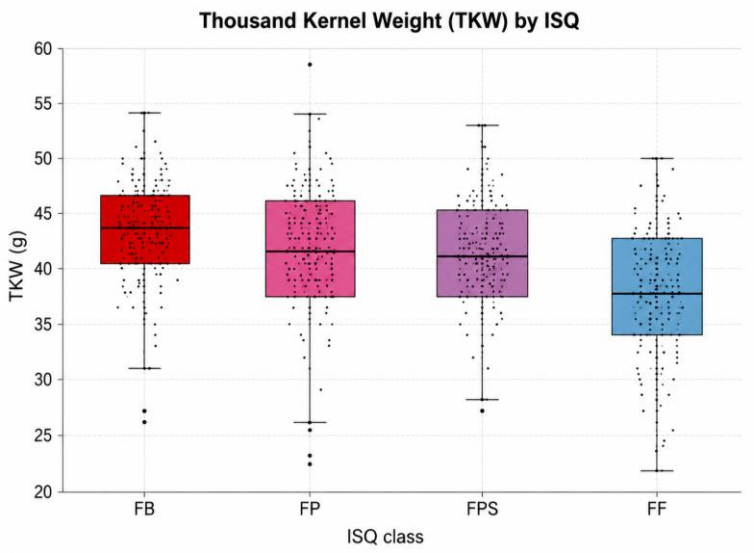

Figure 27: Box plot of thousand kernel weights (TKW) across the ISQ classes (FB, FP, FPS and FF).

These results indicate that TKW was mainly influenced by genotype and growing environment rather than by technological quality class, confirming that kernel size is not necessarily associated with superior breadmaking quality.

4.3 Moisture Content

The boxplot in **Figure 28** represents the distribution of moisture content among the 49 soft wheat varieties evaluated in the National Variety Trial Network. Moisture content exhibited a relatively wide range of variation, with values ranging from 7.73% for the variety BRIGITTA (FP) grown in Piacenza, to 15.07% observed for RGT SCRAMBLER (FP) cultivated in Fiorenzuola d'Arda (PC).

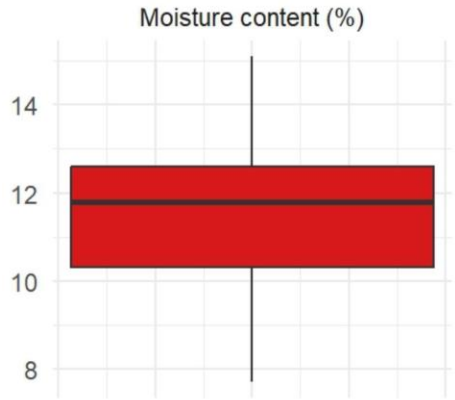

Figure 28: Box plot showing the distribution of moisture content (%) among the 49 soft wheat varieties evaluated in the National Variety Trial Network.

The box plot shows a median moisture content of 11.86%, with 50% of the observations distributed between approximately 10.47% and 12.63%. The overall mean moisture content across all varieties was 11.55%, indicating a generally uniform grain moisture level among the tested materials.

Environmental conditions strongly influenced grain moisture content: when the results were analysed according to location, Voghera (PV) recorded the highest average moisture content (14.20%), while Libertinia (CT) showed the lowest average value (8.14%). These differences reflect the contrasting climatic conditions between Northern and Southern trial sites during the growing season.

Mean moisture values were very similar among the different technological classes **(Figure 29),** hovering around 11.50 %, ranging from 11.51% in the FF group to 11.60% in the FB group. Statistical analysis did not reveal any significant differences among ISQ classes ($P > 0.05$), indicating that moisture content was not associated with the technological quality classification of the varieties.

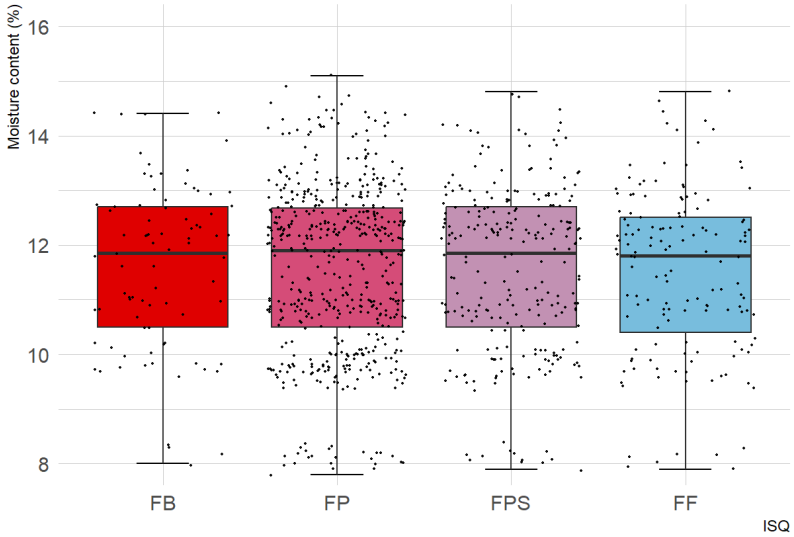

Figure 29: Box plot of moisture content (%) across the ISQ classes (FB, FP, FPS and FF).

4.4 Protein Content

The distribution of protein content among the 49 soft wheat varieties evaluated in the National Variety Trial Network is presented in **Figure 30**. Protein content showed substantial variability, ranging from 8.36% for the variety LG ALVAREZ grown in Idice (BO), to 18.30% observed for RGT IMPAVIDO cultivated in Foggia.

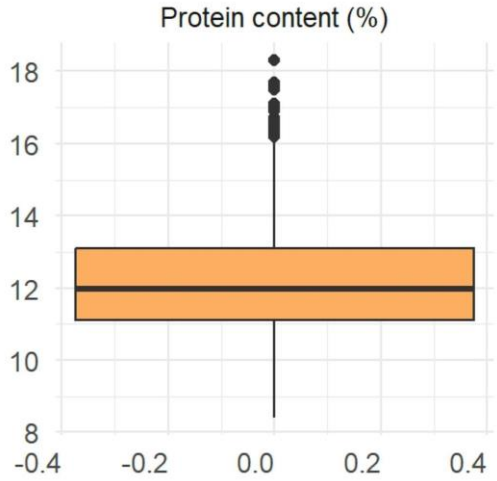

Figure 30: Box plot showing the distribution of protein content (% dry matter) among the 49 soft wheat varieties evaluated in the National Variety Trial Network.

The overall mean protein content was 12.18%, while the median value was 12.10%. The interquartile range extended from 11.10% (25th percentile) to 13.07% (75th percentile), indicating a moderate variability among the tested varieties.

A marked effect of the growing environment on protein accumulation was observed. The lowest average protein content was recorded in Idice (BO), with an average value of 9.61%, followed by Cigliano (VC) (9.72%). In contrast, the highest average protein content was observed in Foggia, reaching 16.04%, followed by Quargnento (AL) (13.59%). These differences highlight the strong influence of environmental conditions on grain protein concentration.

As regards technological quality class **(Figure 31)** , FF class exhibited the highest mean protein content (13.09%), followed by FPS (12.33%), FP (11.93%) and FB (11.77%). This trend reflects the importance of protein concentration as one of the principal parameters used in the classification of wheat varieties according to technological quality.

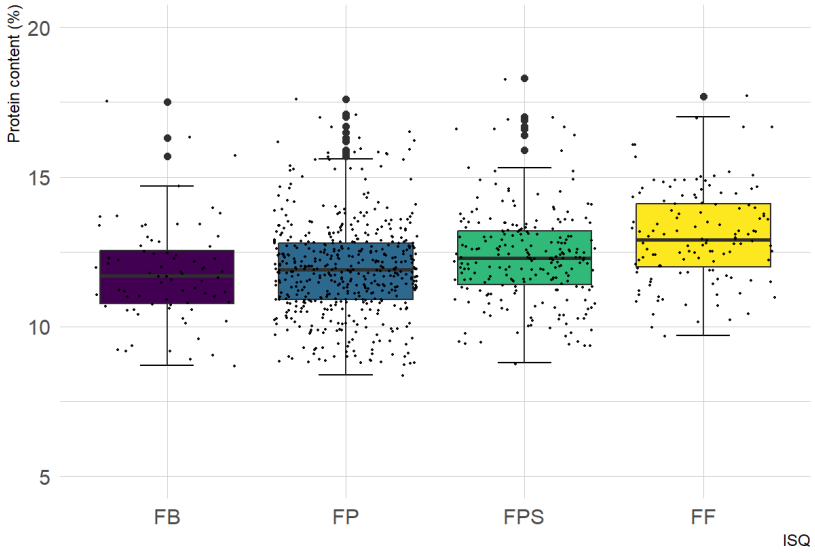

Figure 31: Box plot of protein content (% dry matter) across the ISQ classes (FB, FP, FPS and FF).

The higher protein levels observed in the superior quality classes confirm the central role of grain protein concentration in determining breadmaking potential and ISQ classification.

4.5 Falling Number (FN)

As regards the Falling Number (FN), the average value recorded among the evaluated varieties was 380 sec. FN values ranged from 207 sec to 541 sec.

Only one variety, Atene (FP) grown in Cesa (AR), showed a Falling Number value below the recommended threshold of 220 sec, recording 207 sec. However, this isolated result is not considered particularly concerning, as the vast majority of the evaluated varieties exhibited values well above the critical limit.

Low Falling Number values are typically indicative of pre-harvest sprouting caused by increased α-amylase activity, often associated with excessive rainfall during the final stages of grain

development. The 2024–2025 growing season was characterized by low precipitation across the Italian territory, and this environmental condition was clearly reflected in the Falling Number values recorded during the study.

4.6 SDS Sedimentation Volume (SSV)

The boxplot in **Figure 32** represents the distribution of SDS sedimentation volume (SSV) among the 49 soft wheat varieties. SSV values exhibited high variability: The lowest SSV value was recorded for the variety MODERN (FB) grown in Stezzano (BG), with a sedimentation volume of 32 mL, whereas the highest value was observed for RGT PARKA (FP) cultivated in Alberese (GR), reaching 84 mL.

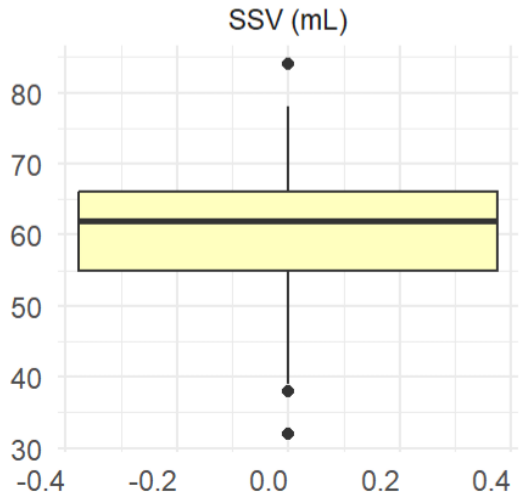

Figure 32: Box plot showing the distribution of SDS sedimentation volume (SSV, mL) among the evaluated soft wheat varieties.

The overall mean SSV was 60.56 mL, while the median value was 62.0 mL. The interquartile range extended from 55.0 mL (25th percentile) to 66.0 mL (75th percentile), indicating that most varieties exhibited medium to high sedimentation volumes.

The growing environment significantly affected sedimentation performance. Among the trial locations, Libertinia (CT) recorded the highest average SSV value (69.57 mL), closely followed by Conselice (RA) (68.59 mL). Conversely, Cigliano (VC) showed the lowest average value (51.22 mL), followed by Stezzano (BG) (52.10 mL).

Higher sedimentation values are typically associated with stronger gluten networks and superior breadmaking performance.

In our trials **(Figure 33)**, the highest average value was observed for FF (65 mL), followed by FPS and FP (62 mL), and FB (59 mL).

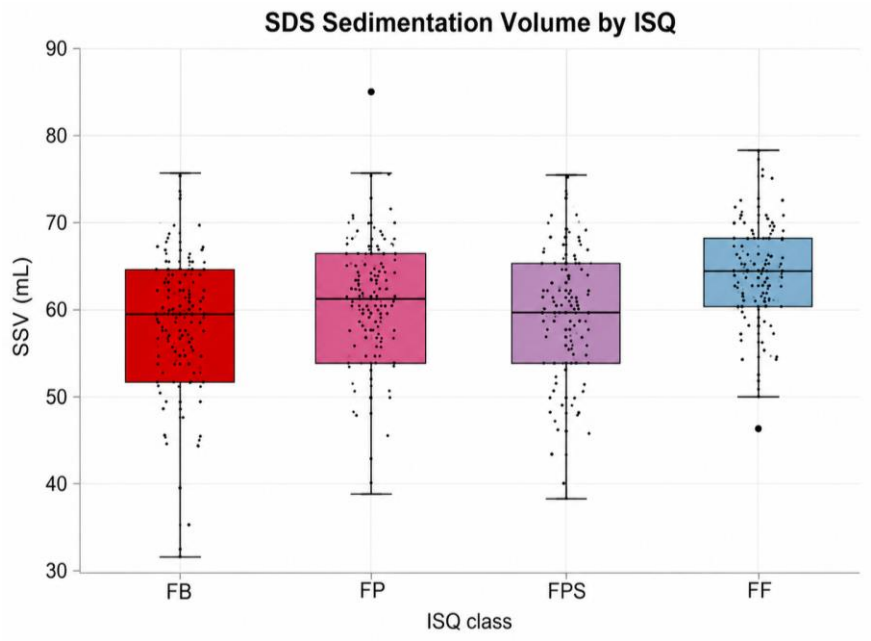

Figure 33: Box plot of SDS sedimentation volume (SSV, mL) across the ISQ classes (FB, FP, FPS and FF).

The distribution of SSV across the technological classes is clearly paralleled to the protein content.

Overall, the results indicate substantial variability in sedimentation volume among varieties and locations, highlighting the combined influence of genotype and environmental conditions on wheat technological quality.

4.7 Deoxynivalenol (DON) Contamination

The distribution of DON contamination among the analysed wheat samples is presented in **Figure 34**. Overall, DON contamination levels were generally low during the 2025 growing season, indicating a favourable sanitary quality of the grain samples.

Most of the observations (69.2%) showed DON concentrations below safety threshold (< 500 μg kg^{-1}). 5.1% of the samples showed moderate contamination (500–1000 μg kg^{-1}). Only a limited number of samples (26) exceeded the European maximum level of 1000 μg kg^{-1} established for unprocessed soft wheat intended for human consumption. Out of these samples, 2.0% showed high contamination (1000 to 1500 μg kg^{-1}), and only three varieties (0.3%) exceeded 1500 μg kg^{-1}.

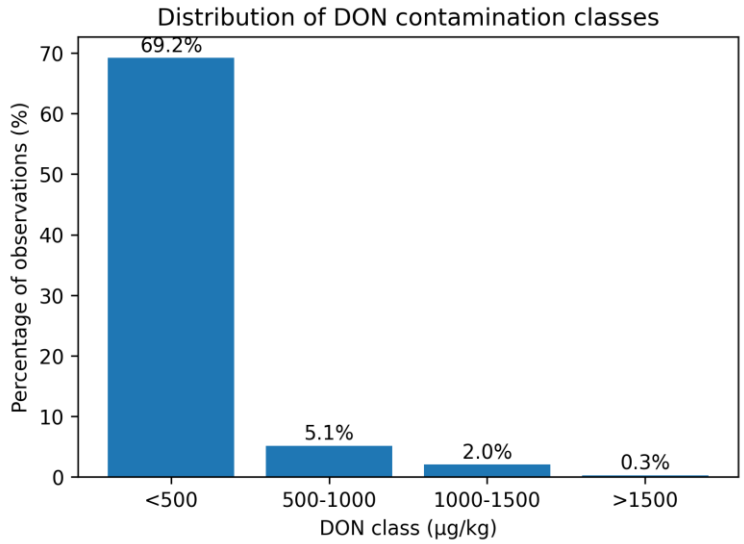

Figure 34: Distribution of DON contamination classes among the analysed wheat samples. Values are expressed as the percentage of observations falling within each contamination class.

Among the trial locations, Lonigo (VI) recorded the highest average DON contamination (492.8 µg kg^{-1}), followed by Piacenza (481.8 µg kg^{-1}) and Stezzano (BG) (470.5 µg kg^{-1}). In contrast, no DON contamination was detected in several southern locations, including Foggia and Libertinia (CT).

Overall, DON contamination during the 2024–2025 season was generally low, with most samples remaining below the European regulatory threshold for unprocessed soft wheat. This result is consistent with the low Fusarium incidence observed during the season, although a limited number of samples exceeded the safety limit and therefore require attention from a sanitary-quality perspective.

4.8 Multivariate Analysis of Quality Traits

4.8.1 Correlation Analysis

A Pearson correlation analysis was performed to evaluate the relationships among the measured grain quality traits **(Figure 35)**. Correlation coefficients ranged from −0.51 to 0.47, indicating generally weak to moderate associations between variables.

The strongest positive correlations were observed between protein content and SDS sedimentation volume (SSV) ($r = 0.47$). As expected, based on the literature, these results indicate that samples with higher protein concentrations tend to exhibit higher sedimentation volumes. Indeed, it is well established that protein content is one of the major factors, together with gluten protein composition, influencing the technological quality of wheat.

A moderate positive correlation was also observed between protein content and test weight (r = 0.46). This moderate positive correlation may be explained by the physiological relationship between protein accumulation and grain development. Under favorable conditions, efficient nitrogen assimilation and translocation to the grain can support both protein synthesis and kernel development, resulting in higher test weight. However, as these processes are also influenced by environmental factors (e.g., water availability, temperature) and genotype, the observed association remains moderate rather than strong

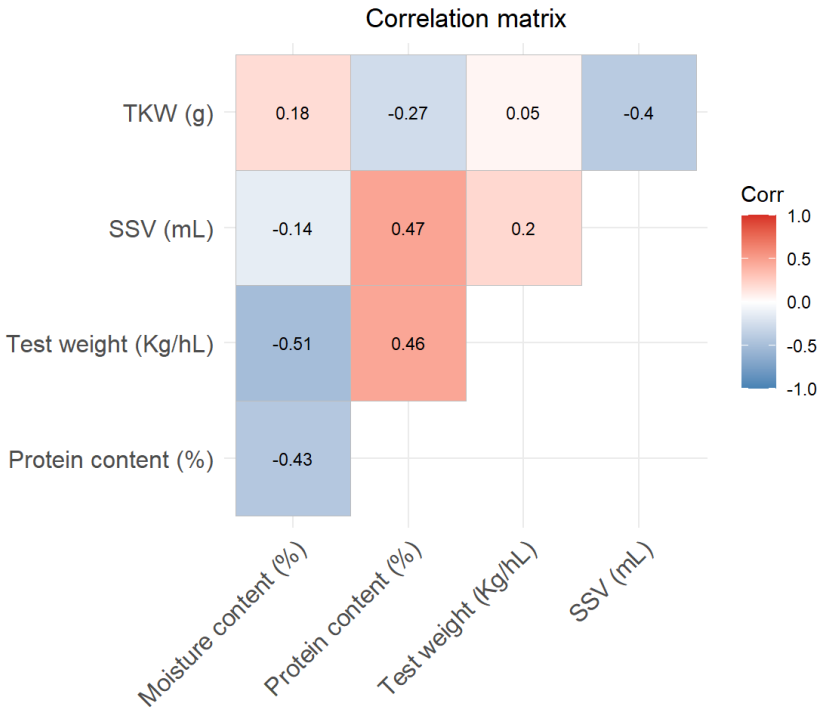

Figure 35: Correlation matrix among the evaluated agronomic and technological quality traits. Positive correlations are shown in blue, negative correlations in red, while the intensity of the color reflects the strength of the correlation coefficient.

In contrast, moisture content showed negative correlations with several technological quality parameters. The strongest negative association was found between moisture content and test weight (r = −0.51), followed by protein content (r = −0.43) and SSV (r = −0.40). These results suggest that samples with higher moisture content generally exhibited lower values for these quality traits. This likely reflects less efficient grain filling and dilution effects associated with higher moisture levels."

The relationship between test weight and SSV was weakly positive (r = 0.20), while the correlation between thousand kernel weight (TKW) and test weight was very weak (r = 0.05). Likewise, TKW showed only a weak positive correlation with moisture content (r = 0.18) and weak negative correlations with protein content (r = −0.27) and SSV (r = −0.14).

4.8.2 Principal Component Analysis (PCA)

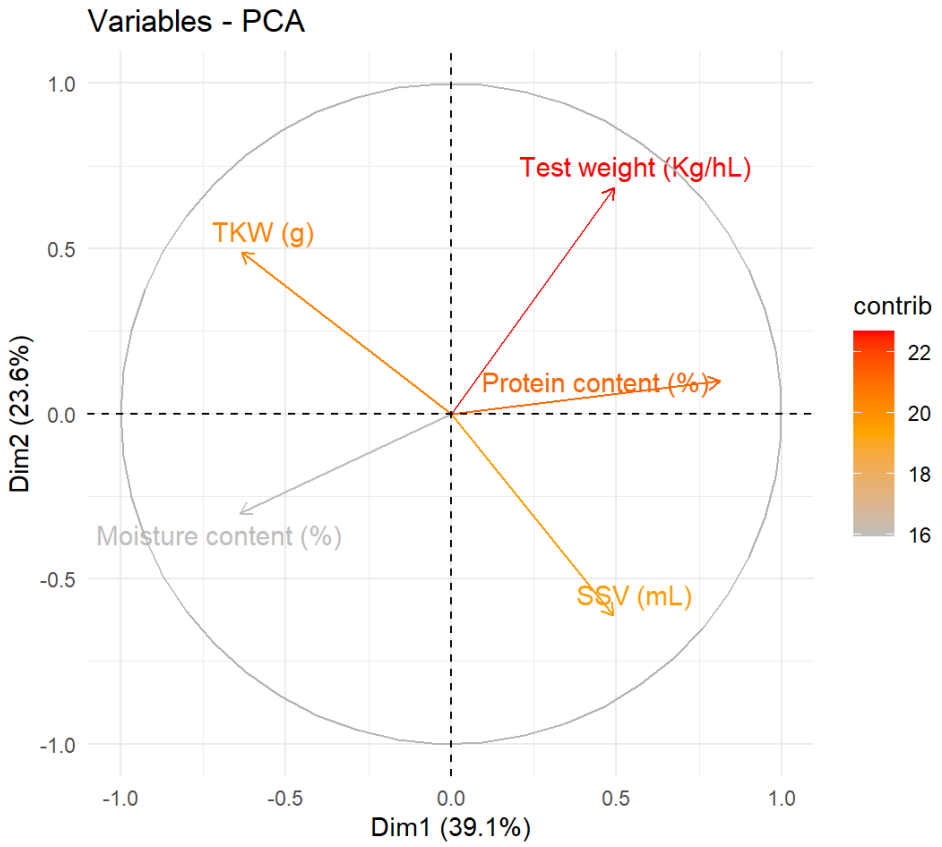

Figure 36: Principal Component Analysis (PCA) showing the relationships among the measured grain quality traits. Vector length indicates the contribution of each variable to the principal components, while the angle between vectors reflects the degree of correlation between traits.

Principal Component Analysis (PCA) was performed to investigate the relationships among the measured grain quality traits and to identify the main sources of variability within the dataset **(Figure 36).** The first two principal components explained 62.7% of the total variance, with Dim1 accounting for 39.1% and Dim2 accounting for 23.6%.

The PCA correlation circle showed that protein content, test weight, and SDS sedimentation volume (SSV) were all positively associated with the first principal component (Dim1), indicating that these traits contributed similarly to the main pattern of variation observed among the samples. Test weight exhibited the highest contribution to the first two dimensions, followed by protein content and SSV.

Moisture content was located on the opposite side of Dim1 relative to the main technological quality traits, confirming its negative association with protein content, test weight, and SSV, as previously observed in the correlation analysis.

Although protein content and SSV were positively associated along Dim1, their separation along Dim2 indicates that sedimentation volume was not exclusively determined by protein concentration, suggesting that additional factors related to gluten composition contributed to the

variability of SSV among samples. Indeed, it is well established that gluten protein composition—particularly the balance of high- and low-molecular-weight glutenins—plays a major role in determining gluten quality.

Thousand kernel weight (TKW) was positioned separately from the other quality traits and was mainly associated with Dim2, indicating that kernel size represents an independent source of variability within the dataset. Overall, the PCA provided a synthetic representation of the relationships among grain quality parameters and confirmed the patterns identified through the correlation analysis.

Overall, the combined use of univariate and multivariate analyses provided a comprehensive overview of the technological quality of the evaluated wheat samples. The distribution of protein content, test weight and Sedimentation Volume, the ISQ classification whereas TKW appeared to be more strongly associated with kernel development and environmental conditions. Correlation analysis and PCA confirmed the positive association among the main technological quality traits and highlighted the negative relationship between moisture content and most quality parameters. These results underline the importance of assessing wheat quality using multiple traits rather than relying on a single analytical parameter.

5. Conclusions

This study evaluated the technological and sanitary quality of bread wheat (*Triticum aestivum* L.) varieties grown within the Italian National Variety Network during the 2024–2025 season. By integrating grain quality traits, technological parameters, and deoxynivalenol (DON) contamination, an overall assessment of varietal performance for the milling and baking industries was achieved.

The results highlighted substantial variability among the evaluated wheat samples for all measured quality parameters, reflecting the combined influence of genotype and growing environment. Moisture content remained within acceptable ranges, confirming the suitability of the harvested grain for storage and processing. Protein content, test weight, thousand kernel weight (TKW), SDS sedimentation volume (SSV), and Falling Number values demonstrated considerable differences among varieties and ISQ quality classes, confirming the effectiveness of these parameters in discriminating technological quality.

As expected, varieties belonging to the higher quality classes generally exhibited superior values for protein content, test weight, and sedimentation volume, confirming the importance of these traits in determining breadmaking potential. The correlation analysis and principal component analysis further emphasized the relationships among quality parameters, showing positive associations between protein content, test weight, and SSV, while moisture content was negatively associated with most technological traits. DON contamination levels were generally low across the tested samples, with most samples well below EU regulatory limits, indicating low sanitary risk for the cereal supply chain during the studied season. The relatively dry conditions characterizing the 2024–2025 growing season likely limited Fusarium development and subsequent mycotoxin production. Overall, the findings demonstrate that the National Variety Network represents an essential tool for evaluating the agronomic, technological, and sanitary performance of bread wheat varieties under Italian growing conditions. The integration of technological quality traits with food safety indicators provides valuable information for breeders, farmers, millers, and the baking industry, supporting variety selection and quality-oriented production strategies.

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Acknowledgements

As I conclude this important chapter of my academic journey, I would like to express my deepest gratitude to all the people who have supported, guided, and encouraged me throughout these years.

First and foremost, I would like to sincerely thank my supervisor, Prof. Giampiero Valè, for giving me the opportunity to carry out this research and for his guidance, valuable advices, and continuous support throughout my master degree course. His expertise, encouragement, and supervision have been fundamental to the completion of this work.

My heartfelt gratitude goes to Dr. Patrizia Vaccino, who has been much more than a co-supervisor throughout this journey. Thank you for believing in me, for your constant support, patience, and encouragement, and for guiding me not only during the preparation of this thesis but also throughout my internship at CREA. Working alongside you has been an invaluable experience. Your professionalism, dedication, and genuine passion for research have inspired me every day. Thank you for sharing your knowledge so generously, for always finding time to help me, and for encouraging me to grow both academically and personally. I will always be grateful for everything you have taught me.

I would also like to express my sincere appreciation to Lorenzo, Simone, and Francesca for their kindness, support, and willingness to help throughout my internship at CREA. Thank you for creating such a welcoming, collaborative, and enjoyable working environment.

To my family, words will never be enough to express how grateful I am. Thank you for your unconditional love, endless sacrifices, and unwavering belief in me. Everything I have achieved has only been possible because of your support.

To Mama, my whole life, thank you for your unconditional love, your endless prayers, your strength, and for always believing in me, even during the moments when I struggled to believe in myself. You have been my greatest source of inspiration, and every success in my life is a reflection of your sacrifices. I hope this achievement makes you as proud as you have always made me.

To my beloved brother, Hamoudi, you are not just my brother-you are my second half, my other soul, and one of the greatest blessings in my life. I cannot imagine my life without you by my side. Thank you for always believing in me, supporting me, making me laugh during the hardest moments, and reminding me that I was never alone. I am incredibly grateful and unbelievably lucky to have you in my life. No matter where life takes us, you will always be my home, my safe place, and my forever best friend. I love you more than words could ever express.

A very special thank you goes to Suzan. Sometimes life unexpectedly brings extraordinary people into our lives, and you have been exactly that for me. You started as a stranger and became family. Throughout these years, you have been my support system, my safe place, and one of the greatest gifts Italy has given me. Thank you for standing by my side through every challenge, for believing in me even when I doubted myself, and for making a foreign country feel like home. Thank you, sister, for always being there. Thank you for being home.

To Elena, the sweetest soul, thank you for your kindness and your beautiful heart. I am incredibly grateful for the coincidence that brought us together. Meeting you has been one of the unexpected blessings of this journey, and I will always cherish our friendship.

To Sadaf, thank you for being such a meaningful part of this journey. I am truly grateful that our paths crossed and that I had the privilege of getting to know you. We have traveled together,

laughed together, cried together, and shared countless unforgettable moments that I will always cherish. Your kind and pure heart has brought so much warmth into my life, and I will forever be thankful for the memories we created together. Thank you for your friendship, your support, and simply for being you. I love you so much.

To my wonderful friends— Giada, Suhail , Sanam, Narges, Fabiana, Ayşegul, Shaghayegh, Raja, Kashi, and all my other friends whom I cannot possibly name one by one (otherwise this page would never end!)—thank you for every laugh, every coffee, every adventure, every word of encouragement, and every memory we created together. You made me feel at home even when I was thousands of kilometers away from my own country. Your friendship has been one of the greatest gifts of these years, and I will carry each of you with me wherever life takes me.

A special thank you goes to Marinella, Ester and everyone at Little House. Thank you for your kindness, generosity, and the warmth you always showed me. Every meal you prepared was much more than food—it brought me comfort, familiarity, and the feeling of being home, even when I was far away from my family. Your care, your smile, and your generosity made a real difference in my life, and I will always remember you with deep gratitude.

Finally, a heartfelt thank you to Vercelli.

Almost 3 years ago, I arrived here carrying dreams, hopes, and many uncertainties. I could never have imagined how much this small city would come to mean to me. Vercelli welcomed me, challenged me, helped me grow, and became the place where I built unforgettable memories, lifelong friendships, and found people who became family.

Here, I discovered not only knowledge and professional growth, but also kindness, love, and a sense of belonging that I will always carry with me. Every street, every corner, and every memory has become part of who I am today.

Although this chapter in Vercelli is coming to an end, I know that my journey in Italy continues. Vercelli will always have a very special place in my heart because it was here that I grew, learned, laughed, cried, achieved my dreams, and became the person I am today.

Thank you, Vercelli, for becoming my first home away from home. You will always feel like home.

To everyone who has been part of this journey, thank you. This achievement is not mine alone—it belongs to every person who believed in me, supported me, encouraged me, and walked beside me along the way.