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How can we evaluate and predict wheat quality?

Valérie Lullien-Pellerin

IATE, Univ. of Montpellier, INRAE, Institut Agro, Montpellier, France

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ABSTRACT

In this review, I would like to summarize some recent innovations in the analysis of wheat quality, illustrated with a few examples. After a brief history of the main targets that have driven selection up to the present day, grain yield, protein content and protein quality, I will explain why studies relating wheat grain characteristics to quality (which appears to be a compromise of many different properties: technological, sanitary, nutritional, sensory, economic) are complex and how the entire production chain may impact the characteristics of the final product. I will then illustrate recent developments that focus on the relationships between grain characteristics and flour properties, notably through non-destructive methods, molecular probes, and "omic" studies, as well as the miniaturization of characterization and processing tools, multi-scale studies and data management. Finally, I will identify possible future directions to better understand, improve, or predict wheat product quality.

1. Introduction

Wheat grains have been essential to human food needs throughout history and currently contribute around 20 % of total human calories consumed globally (Atchison et al., 2010). The domestication of wheat occurred in the Fertile Crescent around 10 000 years ago, with two evolutionary selected mutations distinguishing modern cultivars from wild relatives: these are the loss of ear fragmentation and fragility of the glumes (de Sousa et al., 2021), the latter aiding in the milling of grains into flour and semolina. Since then humans have sought to select the best grains for their use. In France in the 19th century, Pierre Louis François Levêque de Vilmorin (1816–1860, Paris), developed the foundations of wheat genetic selection. Subsequently, the use of genetic selection increased exponentially, notably by Jacques Levêque de Vilmorin (1882–1933, Paris) and especially after the Second World War, in order to adapt and improve wheat to increase production and enhance product quality, primarily for breadmaking. In the 1960s, the number of genes that were selected increased, particularly genes controlling dwarfism to reduce plant height, leading to the Green Revolution to significant increases in the grain yields of commercial wheats (Peng et al., 2011; Balfourier et al., 2019). This strategy spread in global breeding programs alongside a growing interest in the role of specific storage proteins in wheat quality (Kiszonas and Morris, 2018; Shewry and Halford, 2002).

However, the increases in grain yield have been associated with decreases in protein content (Simmonds, 1995), a phenomenon known as "yield dilution". Grain protein content is a major determinant of grain processing quality and therefore the economic value of wheat in the market. It is therefore crucial to understand how protein accumulation is regulated in the wheat grain and how the gluten protein network contributes to the unique rheological properties of the different products made from wheat (Pei et al., 2023; Peng et al., 2022). This is particularly relevant today because of the growing interest in plant-based proteins for human consumption,

However, the study of the relationships between wheat grain characteristics and product quality cannot be limited to rheological properties alone. In fact, product quality is a complex trait, as it includes sanitary, nutritional, sensory and technological properties. It must also be related to a specific product, since different flour properties are required to make bread, pasta, biscuits or cakes. For example, for the traditional French baguette, bakers will select bread wheat grains of medium hardness whereas soft wheat grains are more appropriate for biscuit making. Wheat quality can also be constrained by local legislation, which sometimes limits changes. For example, flour classification in France is based on ash content whereas in UK it is based on flour color. Finally, even if our scientific knowledge of the links between wheat grain characteristics and specific product qualities increases, there are technical and economical limits to their improvement.

The relationships between wheat grain characteristics and final product properties are complex. Firstly, the grain structure and biochemical composition is heterogeneous, consisting of several tissues with different structures and compositions (Evers et al., 1999). In addition, two of these tissues (aleurone layer and the germ) are still live and can therefore respond metabolically to changes in the mature grain

E-mail address: valerie.lullien-pellerin@inrae.fr.

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environment, notably the water content. Grains are also heterogeneous in size, shape and degree of maturity depending on their position in the ear (Baranova et al., 2022; Huebner et al., 1990). Grain structure and composition also reflect both wheat genetics and the effects of environmental and agronomic conditions (soil, temperature, precipitation, fertilizers, insect and fungi attacks, agrochemical treatments). The grains are then processed by millers into flour and semolina, which are mainly used to make products for human consumption, and bran and shorts, which are mainly used for livestock feed or non-food purposes. The effects of processing on the different grain tissues will also depend on the processing conditions, in particular the water content of the grain, the sequence of operations (eg. peeling, abrasion, grinding, sieving) and the equipment used (eg. stone mill, cylinder mill, jet-mill), but also the grain characteristics (size, shape, hardness). For example, the fate of the aleurone layer in wheat grains varies as a function of grain hardness (Greffeuille et al., 2005; Heinze et al., 2016). Although the starchy endosperm is the major tissue recovered in white flour obtained from milling of common wheat grains, the presence of other tissues, and in particular the aleurone layer, may change the final flour properties. The aleurone layer may contain active enzymes as well as a number of micronutrients (minerals, B vitamins) and fiber, as well as proteins enriched in essential amino acids (notably lysine) which may increase the nutritional value of the flour, although also contains phytic acid which may be considered either as an antinutrient or as a beneficial compound (Lullien-Pellerin, 2021). Finally, flours may be hydrated with only water but also with additives, improvers, ingredients, yeast and enzymes, depending on the recipes, using different equipment and protocols according to the type of operators (artisanal or industrial) and transformed into different products with specific properties. Thus, the entire chain itinerary impacts the final product quality.

This review aims to illustrate and discuss some of the recent innovations in the characterization of grain and flour traits in relation to the quality of wheat products. In particular, the development of non-destructive methods, molecular probes, "omic" studies, and the miniaturization of tools to determine wheat quality on small samples. It will also emphasize the importance of studying grain at different scales, from micro to macro, both by acquiring experimental data and using numerical modelling. It will also discuss new methods of data management that will speed up information processing and make it easier to establish links between diverse and scattered data.

2. Selection of wheat grains or milling products free from insects, pathogenic fungi and undesirable compounds

2.1. Detection of insects

Wheat grain losses, particularly during storage, are mainly caused by two different orders of insect: *i.e.* the Coleoptera (beetles) which include for example *Sitophilus oryzae*, *Sitophilus granaries*, *Tribolium castaneum*, *Tribolium confusum*, *Trogoderma granarium*, and *Oryzaephilus mercator* and the Lepidoptera (moths and butterflies), such as *Plodia interpunctella*. Infestation results in grain damage or the presence of undesirable compounds. Insects may be present in grains in different forms (adults, pupae, larvae, eggs) and in different states (dead or alive). Therefore, methods for characterizing infected grains have to take this diversity into account in order to assess the level of contamination which may affect both production and product quality. They also need to be applied quickly when the insect presence is suspected to avoid further grain degradation over time and the spread to other uncontaminated grain samples. Progress in the development of innovative methods was recently reviewed by Zhu et al. (2022).

Two types approaches were described that may be applied to grains or flours:

Non-destructive methods include direct insect detection, such as machine vision, which mimics the human eye and allows pests to be identified by their morphology after image capture and processing, aided by various databases and learning algorithms or acoustic methods, which detect insects through their feeding activity. However, machine vision is inefficient for the detection of hidden insect whereas acoustic methods will not detect dead insects or eggs and are very sensitive to environmental noise, which limits their use to confined environments. The indirect detection of insects is also possible by identifying volatile metabolites that they produce with an electronic nose.

Destructive methods are based on extracting DNA and using a panel of molecular techniques such as the use of DNA barcoding or qPCR, particularly targeting the insect mitochondrial cytochrome oxidase subunit I gene. However, these methods are unable to distinguish the different forms of insects.

Thus, there are no ideal methods to reveal the presence of insects and one has to choose the most relevant depending on the material, resources and objectives.

2.2. Sorting grains with low level of contaminating fungi and mycotoxins

Infection of wheat grain with mycotoxigenic fungi such as *Fusarium* may also cause significant losses or lead to rejection of wheat grain samples. Different strategies have been developed to identify contaminated grains and even to sort out the infected grains based on differences in the spectral response at distinctive wavelengths. Indeed, NIRS, multispectral and hyper-spectral imaging (HSI) were recently developed to identify and classify wheat samples according to the level of grain damage through statistical data treatment (Caporaso et al., 2018a; Jaillais et al., 2015; Peiris et al., 2012). There are fast and non-destructive methods able to determine properties such as water and protein content of single object that can be grains, grain sections, tissues, particles (flour, semolina) or even products. Multispectral and HSI methods in particular provide both spatial and spectral information at different wavelengths, and especially in the near-IR, through transmittance/reflectance or fluorescence and were particularly effective in visualising the location and intensity of the changes resulting from fungi infection. However, to compare samples and relate spectral response to the fungi infection, it is necessary to prepare samples under identical conditions and to cross spectral data with more classical method such as qPCR detection of *Fusarium* specific genes. The accuracy of these new methods then needs to be confirmed using another set of unknown samples.

Concomitantly with the detection of mycotoxigenic fungi, new approaches (Santana Oliveira et al., 2019; Zhang et al., 2023b) were used to substitute the time-consuming and high cost analysis of mycotoxins produced by the pests by high-performance liquid chromatography or gas chromatography coupled with mass spectrometry analysis. Although immunoassays are being continuously improved, they are mainly qualitative or semi-quantitative with low reproducibility and sensitivity and can give to false-positive results. By contrast, bio-sensors being developed for the detection of specific molecules are sensitive, low cost and may even be developed in mobile versions. These bio-sensors consist of a sensing surface that specifically interacts with the molecule of interest, a transducer that converts the interaction into a signal that can be processed, and a signal processing system that amplifies the signal and displays it in an appropriate format, resulting in high sensitivity and selectivity. Different molecules may serve as efficient detectors of mycotoxin and their diversity was recently reviewed (Santana Oliveira et al., 2019; Zhang et al., 2023b). However, the use of aptamers should be highlighted as a growing and very sensitive method for the detection of mycotoxins. This involves the selection of a single RNA or DNA sequence which is able to recognize the target molecule according to its molecular folding from a large bank of combinatorial sequences (Fig. 1).

This approach was shown to be effective for rapid detection of low amount of mycotoxins with a detection limit of between 2 and 340 pM depending on toxin: T-2 toxin (Khan et al., 2018), ochratoxin (Lv et al., 2023), zearalenone (Ma et al., 2023) or deoxynivalenol (Yu et al., 2023).

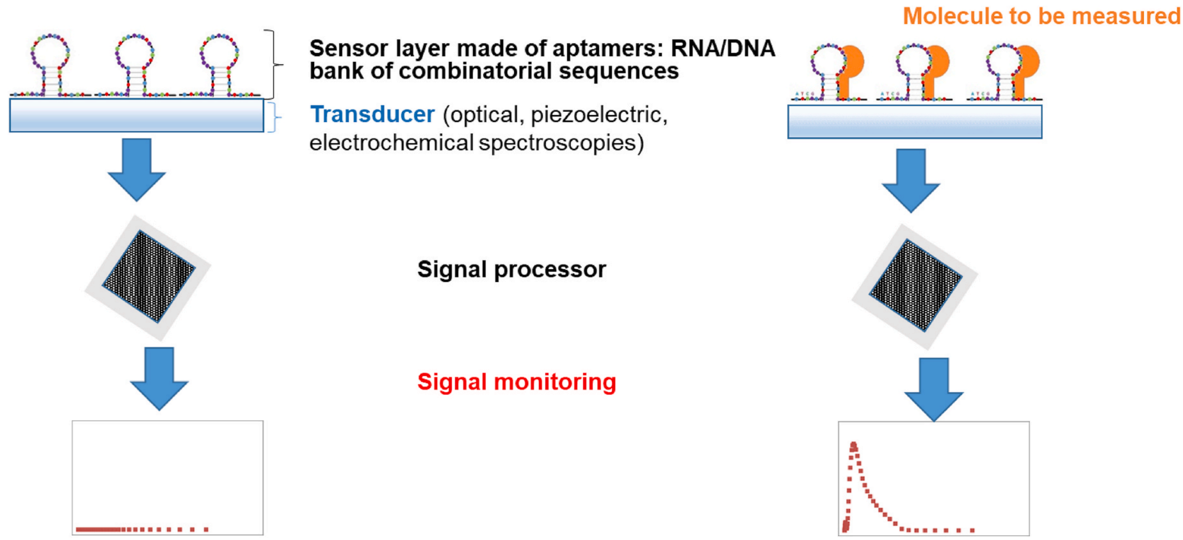

Fig. 1. Schematic representation of a biosensor using aptamers as the sensor able to capture the molecule to be measured and additional equipments for monitoring and measuring the signal of the complex between the aptamer and the targeted molecule.

3. New development to characterize wheat grain structure, mechanical properties and biochemical composition

3.1. Non-destructive methods to study wheat grain structure and biochemical composition

Different applications of HSI (reviewed by Caporaso et al., 2018a) have been developed to classify cereal grains depending on their microstructure (*i.e.* porosity, either vitreous or mealy (Lullien-Pellerin, 2020)) or for the identification of sprouted grain, which leads to changes in composition. HIS has also been used to monitor the water uptake or water content of grains, or to predict the amounts of specific biochemical compound in grain, flour or semolina with the advantage of being a non-destructive method. As an example, HSI was found efficient to predict the protein content in each individual wheat grain showing variation within a bulk sample (Caporaso et al., 2018b). It was also shown to be efficient for predicting the amounts of different molecules such as protein, starch or water in wheat flour (Zhang et al., 2023a) and to quantify the fiber content in semolina or in the final pasta product (Badaro et al., 2019; Badaró et al., 2021).

Non-destructive grain structure analysis has also been made using X-ray micro-tomography but is time consuming as high resolution and the analysis of a number of grains are required (Besançon et al., 2020).

3.2. Other methods to study wheat grain structure from the grain to the molecular scale

Recently, destructive analysis has been used to provide objective data to better characterize the porosity of the inner starchy endosperm, its distribution within the grain and differences between samples grown in different locations (Chichti et al., 2018). The use of a specific assembly composed of a plate light diffusing uniform white LED lighting underneath the examined sample, an optical fiber and a spectrometer, allowed light transmission to be measured through a wheat grain section of controlled thickness (Fig. 2). This showed that the level of porosity of the endosperm microstructure followed the Beer-Lambert law. These differences in grain porosity are particularly important as they are related to environmental conditions (Oury et al., 2015). Moreover, both wheat grain porosity (i.e. the inverse of vitreousness) and hardness were found to have impacts on grain milling behavior (Oury et al., 2017).

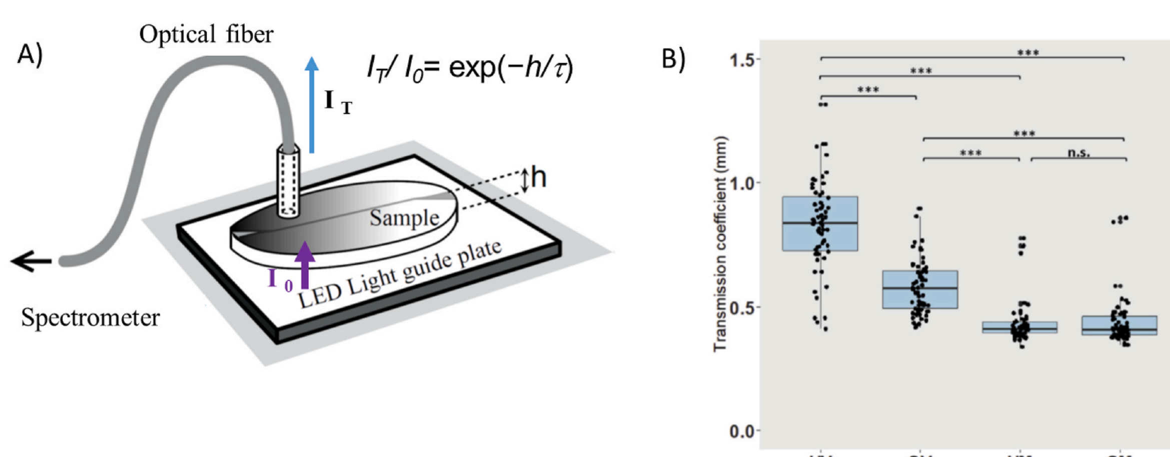

Fig. 2. A) Schematic representation of the device assembly to measure the light transmission I_T through wheat grain sample slices of controlled thickness (h); B) Boxplot for comparison between transmission coefficient (τ) measured within grains from four distinct wheat samples (n = 180 measurements within each wheat sample). HV and SV or HM and SM corresponds to wheat near-isogenic lines differing by the gene encoding one of the PINs (PINB) leading to contrasted hardness: H, hard or S, soft. HV and SV differ from HM and SM by the growning location which leads to changes in the endosperm microstructure, *ie.* porosity (for details see Chichti et al., 2018).

Considering grain hardness, Barlow et al. (1973) demonstrated the importance of the interface between starch granules and the protein network as opposed to differences in the mechanical properties of the constituting polymers. The *Ha* (for hardness) locus on the short arm of chromosome 5D was shown to be the main genomic contributor (Law et al., 1978) encoding two specific proteins, the so-called puroindolines (PINs), which were proposed to play a role at the starch-protein interface (Greenwell et al., 1986; Morris, 2002). More recently, Chichti et al. (2013) used the tip of an atomic force microscopy (AFM) to determine the hardness of extracted starch and gluten, polymers which have similar properties either as extracted material packed into tablets or inside the grain. The mechanical resistance of starch was found to be about 2.4 GPa, a value close to calcite (=3 GPa) and three times higher than that of gluten (around 0.7 GPa) which was closer to that of talc (=1 GPa), the softest material on the Mohs scale. In addition, AFM was also used to determine the properties of the surfaces of starch graules, after using the AFM tip for abrasion, in cryo-sections of grain of near-isogenic lines carrying either only the wild-type PINs, and exhibiting a soft phenotype, or having a mutation in one of the PINs, and thus exhibiting a hard phenotype. This showed differences in the starch-protein interface consistent with the role of PINs in reducing adhesion between these two polymers (Chichti et al., 2015).

Numerical models have been developed using data from the mechanical properties of the major polymers and different levels of adhesion at the polymer interface to mimic endosperm structure and mechanical resistance in order to predict grain fracture during processing. The new experimental data obtained for the hardness of starch and gluten polymers questions previous established numerical models built with values reported by Barlow et al. (1973) or Glenn and Johnston (1992) who reported similar values, around 0.3–0.5 GPa, for both polymers (which is not consistent with those reported by Chichti et al. (2013). The previous values were obtained by the indentation of the endosperm surface but because the surface of the starch granule is generally coated with gluten it is only possible to determine the properties after abrasion with the AFM. Considering these data, a new numerical model, taking into account the variability of the protein matrix amount, starch granular assembly and different levels of adhesion to mimic the soft and hard classes, was developed (Chichti et al., 2016).

This model was found to accurately reflect the fracture behavior observed for samples of starchy endosperm (Fig. 3) with contrasting origins: *i.e.* from wheat near isogenic lines differing in wild-type or mutated PINs and differing in compactness (mealy or vitreous or grown in different locations). Moreover, the observed differences in the mechanical behavior of endosperm sample were related to (1) the energy required to obtain flour at first grinding, (2) the amount of flour produced, and (3) the degree of starch damage. However, the numerical models to predict the grain milling behavior still need to be refined to take into account grain 3D structure and the contributions of other tissues.

3.3. Down-scaling of the milling process for earlier and faster selection of the best wheat samples

Miniaturization of the milling process using simplified milling diagrams with a reduced number of operating units were recently reported to predict semolina yield using only 100–200 g of durum wheat grains (Sissons et al., 2020; Wang et al., 2019). Depending on the conditions, it was also shown to produce semolina with similar levels of some components, such as ash, protein and yellow pigment, and similar quality criteria such as wet gluten and gluten index. Nevertheless, the robustness of these diagrams needs to be tested with a larger panel of wheat samples with different genetic backgrounds and environmental origins

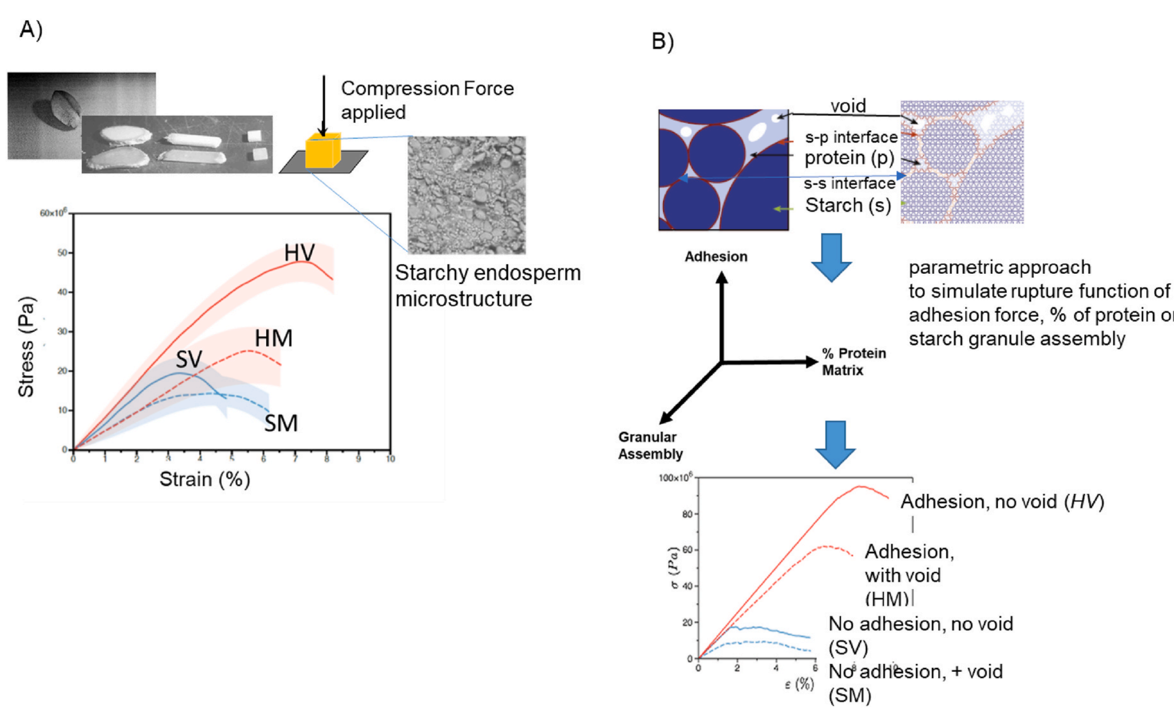

Fig. 3. A) Experimental measurement of the starchy endosperm mechanical resistance: parralelic cubes inside the endosperm were obtained by manual abrasion within four contrasted wheat samples (same as in Fig. 2). A compression force was applied on these samples and stress-strain curves reported. B) 2D numerical modelling was performed (Chichti et al., 2016), using a lattice element method based on the starchy endosperm microstructure taking into account the respective protein and starch mechanical resistance as previously determined (Chichti et al., 2013), absence or presence of void to respectively mimic the vitreous or porous character of the starchy endosperm, different proportion of starch granules and the protein matrix, and different adhesion forces to mimic soft wheats (lacking adhesion at the interface between starch and the protein network) or hard wheats (with adhesion at the interface between starch and the protein network). Mechanical rupture curves were obtained depending on the microstructure using the constructed numerical model and found to exhibit similar profiles as the experimental ones.

and compared with more complex industrial mills. For this purpose, a new device was developed and patented conjointly by Chopin Technologies (now KPM analytics), INRA (now INRAE) and Arvalis (Dubat et al., 2015) to evaluate milling of bread wheat. This was shown to be capable of measuring the milling behavior of wheat samples grown with different agronomic and environmental conditions with a small amount of grains and predict total flour yield (Oury et al., 2017). Nevertheless, the amount of grain required to perform these simplified milling systems is still too high for the system to be used to select wheat with a better milling properties earlier in the breeding process.

4. Omics studies related to quality traits

In recent years, research has been intensified to correlate the genomic expression of wheat with the product quality, in particular, to increase the amount of nutritionally beneficial compounds and to reduce those that are potentially harmful to health. To better understand the regulation of the deposition of beneficial nutrients, a combination of transcriptomic and metabolomic analyses was used to identify the differences between wheat grain tissues and developmental stages (Zhi et al., 2023). Important genomic regions and key candidate genes affecting grain quality traits such as test weight, and protein or gluten content have also been identified using meta QTL analysis combined with a transcriptomic analyses (Li et al., 2023). Similarly, proteomic studies were also used to characterize differences between flours with different technological properties (Victorio et al., 2021) and to determine the effect of environmental conditions on the protein expression including allergenic proteins (Afzal et al., 2023). Lipidomic studies have also revealed differences in flour or dough quality associated with allelic variation at a QTL for loaf volume (Min et al., 2020). Finally, ionomics have been used to determine the effects of genotype, environment and agronomy on the contents of mineral micronutrients (Sinto et al., 2022).

5. What might be the future goals?

While some progress has been made in recent years to more rapidly assess grain properties, including safety, and their relationship with grain fractionation behavior, there has been little success in relating these data to final product quality. This is firstly due to the difficulty of predicting the rheological behavior of gluten, which underpins processing quality, but also mainly due to the multi-factorial nature of product quality. Recent developments in artificial intelligence can speed up the construction of decision support systems capable of integrating and relating heterogeneous data and knowledge, either from experiments, but also from the literature or experience, under a common ontology to better predict product quality and possibly facilitate the design of products with desired qualities. Indeed, new approaches such as neural networks, Bayesian statistics and machine learning allow increases in the number of data obtained when working with a large range of resources and equipment and at different scales or of dimensions and from distinct disciplines. The expected outputs of such decision support systems will need to respond to the general objective, which is to maintain the product sensory and safety qualities, while increasing nutritional quality and reducing components that may result in adverse effects. At the same time, new factors must be taken into account, such as the impact of climate change on grain characteristics, changes in agronomic practices to reduce environmental impacts, and the reduction of energy and water used in each processing step to construct a more durable wheat chain. Interactions between researchers from different disciplines, as well as stakeholders of the wheat value chain, will need to work together to meet these challenges.

CRediT authorship contribution statement

Valérie Lullien-Pellerin: Writing – review & editing, Conceptualization.

Declaration of competing interest

I declare that I have no competing financial interests or personal relationships that could influence the work reported in the submitted article entitled "How can we evaluate and predict wheat quality? For Journal of Cereal Science.

Data availability

No data was used for the research described in the article.

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