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Innovations and improvements in flours production chains: a focus on machinery and plants

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Abstract

Bread and bakery products are considered worldwide as essential food for human nutrition. They are a fundamental source of energy, protein, dietary fiber, vitamins, micronutrients, and antioxidants. The recent increase in consumer interest observed for bakery products able to provide health benefits by means of bioactive compounds, has led to an important focus on the rediscover of ancient wheats and in the use of whole wheat flours in the food industry.

Despite a more suitable nutritional profile, the ancient wheats and whole wheat flours are characterized by worse rheological properties of doughs and lower bread volume, compared to doughs and bread obtained by modern cultivars. This highlights the need for technological innovations and improvements strategies, from cradle to grave, for the entire production chain, thus motivating the aims of this thesis.

The aims of this thesis are to provide technological innovations and improvement strategies, with an approach “from cradle to grave”, to the production chains of flours and bakery products. In order to provide technical solutions and significant ameliorations for farmers, food producers, and food industry, these improvements needs to be focused on the main operations of the production chains (i.e. wheat cultivation, wheat milling, dough processing, and finally, the manufacturing of bread and bakery products). For this reason, the research activities were divided into three main operational phases: *Ex-ante* (focused on innovations and improvements in field), *Inter* (which regards innovations and improvements related to machinery, plants, processes, and doughs rheology), and *Ex-post* (focused on innovations and improvements linked to the sustainability of flours, pasta, and bakery products).

Starting from Ex-ante (i.e. from the field), the results suggest that by means of the correct management of the agronomical treatments, such as nitrogen and sulfur fertilizations, it is possible to significantly improve the nutritional content and the technological performances of wheat kernels, flours, dough, and bread. This strategy appeared particularly effective for the technological improvement of ancient wheats flours and bread, however, it has produced excellent results also for unrefined flours like type 2 and whole wheat flours. With respect to the Inter operational phase, this highlighted that through the correct management of the milling process is possible to improve both ancient wheats and whole wheat flours. In particular, by the use of stone mill, making sure to maintain low milling temperature and stone rotational speeds, is possible to increase significantly the nutritional content of both ancient wheats and whole wheat flours. Moreover, by the correct management of wheat conditioning (optimal moisture content 13-14% for soft wheat) is possible to improve significantly flour yield, flour quality, dough rheological properties, and bread characteristics.

Regarding innovations and improvement strategies for roller milling, the wheat debranning before milling seems to be the most promising innovation. This is particular interesting when bran, middlings, and germ were stabilized with new technologies such as light steam treatments, microwaves, or infrared radiation, because this approach allows to stabilize and store separately the non-endospermic components of wheat (i.e. bran, middlings, and germ) that will be successively reinserted into the refined flour, thus facilitating the management of whole wheat flours orders, increasing whole wheat flour shelf life, and improving the nutritional characteristics of whole wheat bread. Another improvement strategy for roller milling use the break system and the sizing and reduction systems of the roller mill for flour differentiation. The results demonstrate that the different sections of the roller mill (i.e. break, sizing, and reduction systems) could be used to produce flours with different nutritional content and technological properties starting from the same batch of wheat, without any additional cost and without extending the duration of the milling process.

Proceeding in the Inter operational phase, this step also provide innovations and improvement strategies for dough rheology and dough kneading. In particular by means of the correct management of the total water content in doughs it is possible to improve refined ancient wheats flours, type 2 flours, and whole wheat flours. Moreover, to significantly improve the kneading phase, it appear to be essential to control the evolution of dough temperature and the correct dosage of the ingredients. In this direction, two specific innovations were provided by this thesis. The first concern the assessment of the effects related to the addition of CO_2 snow

during kneading on thermoregulation, dough rheological properties, and bread characteristics. The results clearly show that high percentages of CO_2 snow (6%, 8%, and 10%) are able to avoid dough warming during kneading and to significantly improve bread characteristics, in particular bread volume, both in ancient wheats and modern wheats flours. The second innovation proposed in the Inter operational phase was specifically developed for improving whole wheats flours, doughs, and bread. The idea of this innovative strategy is to delaying the addition of bran and middlings during kneading to guarantee a correct gluten development and improved whole wheat bread characteristics. The results shows that a delay of 2 min (25% of the total kneading time) improved dough rheology and whole wheat bread characteristics, highlighting the interesting possibility to develop specific kneading machines for the whole wheat flours.

Last but not the least, the Ex-post operational phase. This step focused on the increase of flours, pasta, and bakery products sustainability through the reduction of the environmental pressures by assessing the effectiveness of alternative sources of proteins and through the LCA analysis. Regarding the effectiveness of these alternative sources of protein, in the comparison between the tested insects flours, cricket flour displayed better rheological and technological properties than those obtained for *Tenebrio molitor*. Nevertheless, only the substitution with 5% chickpea flour proved to be a significant improver for ancient wheats flours, dough and bread. Another way to reduce the environmental impacts of the flours production chains is to assess, through LCA analysis, the impacts related to each single process of the supply chain. As shown in the results of this thesis, through the separate evaluation of the environmental impacts related to each process of the production chain, it is possible to provide specific improvement strategies that can lead to a significant reduction of the environmental pressures.

1 Introduction

1.1 Flour production chain: the strategic relevance of the sector

Bread and bakery products are considered worldwide as essential food for human nutrition (Cappelli et al., 2018). They are a fundamental source of energy, protein, dietary fiber, vitamins, micronutrients, and antioxidants (Boyacioglu & D'Appolonia, 1994). The centrality of wheat and flour production chains reflects the essentiality of bread and bakery products in world populations diets. In particular, this production chain hold the second position in Italy, in terms of sales values, with 12.9 billions of euros (UniCredit, 2016).

Fig 1: Production of Triticum aestivum and Triticum durum with the pertaining yields in soft wheat flour and durum wheat semolina (Italy). (Source: Igiene Alimenti, February-March 2020).

In Italy, every year, 5,410,000 tons of *Triticum aestivum* and 5,660,000 tons of *Triticum durum* are cultivated, harvested and processed to produce wheat flour and wheat semolina, respectively. The respective production of 4,005,000 tons of soft wheat flour and of 3,773,500 tons of durum wheat semolina, is an essential base for Italian food production companies which, often, process only Italian ingredients to be able to boast a caption "100% Italian product", with potential benefit in products marketability. In Italy, the processing of raw materials and the production of bread and bakery products, is mainly performed by small and medium-sized companies, which are, without any doubt, the ambassadors of Italian food excellence. On the other hand, several multinational food corporations, Italian and not, have their base of operations in Italy. The activity and the business volume of the various multinational food corporations in Italy must not be neglected, since it covers a very significant percentage of the overall business volume of the Italian bakery industry. Despite Italy also purchase wheat and flour from others countries, the internal wheat and flour production, represent the most valuable resource for the production of pasta, bread, and bakery products, destined both to export and internal consumption. Notwithstanding in Italy and in others country the expenditure for the purchase of food has been reduced due to economic crisis, it seems that the consumers do not renounce to food quality, with potential benefit to niche products like ancient wheats and local productions. Recently, a significant increase in consumer interest has been observed for bakery products that are able to offer health benefits by means of bioactive compounds (Van Kleef et al., 2018). This has led to the revival of the cultivation and use of ancient wheats and of whole wheat flours in the food industry (Schmiele et al., 2012).

1.2 Need for innovations and improvement strategies for ancient wheats and whole wheat flours

The recent increase in consumer interest observed for bakery products able to provide health benefits by means of bioactive compounds, has led to an important focus on the rediscover of ancient wheats and in the use of whole wheat flours in the food industry (Cappelli et al., 2018; Schmiele et al., 2012). Regarding ancient wheats, the revival of the cultivation and use of several ancient local varieties, has contributed to the safeguarding of biodiversity (Cappelli, 2020; Recchia et al., 2019). This is a topic that is particularly relevant in Italy, a country that is considered worldwide to be a treasure chest of biodiversity (Cappelli et al., 2018). Moreover, it has permitted the development of a local micro-economy, which is continuously growing, and which allows local producers to differentiate their products and increase their remuneration. The term ancient grains is used in literature for either old *Triticum aestivum L*. varieties or wheat relatives such as spelt. This has generated confusion both in researchers and in consumers, who do not clearly know what is meant by this term.

In fact, nowadays, the term ancient wheats divides the scientific world about several aspects, in particular regarding what is meant by this term. If a literal definition of the term was used, only ancestral or very old wheats might be included in the ancient wheats category (Cappelli, 2020; Cappelli & Cini, 2020c). In this way, the only one that can be definable as “ancient” would be the *Triticum monococcum*, which was the first domesticated and cultivated species of the genus *Triticum*. Furthermore, in various scientific publications, additional terms like “old wheat varieties” can be found (Cappelli, 2020; Cappelli & Cini, 2020c). This might generate further confusion in both readers and researchers. But does all this make sense? Does it make sense to define ancient wheats according to a temporal basis constructed on such an ancestral watershed? Definitely no (Cappelli & Cini, 2020c). In fact, Highlighting the lack in the literature of an unambiguous definition of ancient wheats, with a clear, unequivocal, and univocal meaning, one of the aims of this thesis is to provide a definition of ancient wheats which finally provides a clarification on this important topic.

Some authors tried to define which is the boundary that separates ancient and modern wheats. In the literature it is possible to find several proposals. The most "curious" classifications are based on historical dates and events; for example, in one of these, ancient wheats were defined as those varieties existing before the First World War, and modern wheats, instead, were those varieties developed after the First World War. These authors have chosen the First World War as watershed, as if it had been the First World War to produce the most significant changes in wheat. Based on what reported in the literature, we can only disagree (Cappelli, 2020; Cappelli & Cini, 2020c).

However, it is essential to correctly define what is meant with the term "ancient" through the definition of a specific temporal cut-off which have an unambiguous sense. In particular, it is necessary to select the watershed that has determined the most significant changes in wheat characteristics. These changes must be so deep and significant to justify that after that moment the wheat will be completely different than before. It seems obvious that the most suitable cut-off is the green revolution (Cappelli, 2020; Cappelli & Cini, 2020c). Why the most suitable? Because the American plant breeders’ approach of the XX century has determined the most significant changes in wheat.

Precisely, before this approach, the large-sized varieties were considered as the most suitable and the ones capable of guaranteeing high productivity, after which, instead, the most productive varieties became the semi-dwarf ones (Cappelli, 2020). Before the green revolution, the varieties were characterized by higher adaptability and therefore by reduced input of fertilizers and chemicals. Successively, on the contrary, the wheat varieties were characterized by lower adaptability and by the need for high inputs of fertilizers and chemicals (Cappelli, 2020). Another important difference is related to the grain and straw yields, which ex-ante were high for straw (which, for example, had a great economic value in Italian agriculture) and good for grain, while ex-post, became low for the straw and from high to very high for the grain. Not to mention the significant differences in terms of "genetic erosion" and biodiversity protection between ancient and modern wheats (i.e. between plant breeding approaches prior and after the green revolution) (Cappelli, 2020). In conclusion, all these aspects highlight that the deepest changes in the characteristics and in the techniques of wheat cultivation are due to the green revolution (Cappelli, 2020).

Therefore, which might be the correct definition of ancient wheats? Bordes et al. (2008) provided a definition of modern wheats by defining them as those cultivars developed after 1960. Unlike these authors, who use this date as the watershed which divides ancient and modern varieties, is preferable to use the year 1961, since it corresponds to the release date of the first wheat cultivar definable as semi-dwarf (i.e. Gaines 61) released by Orville Vogel (Cappelli, 2020; Cappelli & Cini, 2020c). This clarification, which may appear to be punctilious, allows instead to include in the ancient wheats category all the varieties released in the year 1960, such as the Italian cultivar Sieve, which otherwise would risk the exclusion from this classification (Cappelli, 2020; Cappelli & Cini, 2020c).

In conclusion, a proper definition of ancient wheats might be: “The term ancient wheats refers to all those wheat varieties (genus *Triticum*) not subjected to intensive genetic improvement programs and characterized by an origin prior to the year 1961" (Cappelli, 2020; Cappelli & Cini, 2020c). This new and precise definition includes spelt (*Triticum spelta, Triticum monococcum, and Triticum dicoccum*) in ancient wheats. Nonetheless, pseudocereals like amaranth, buckwheat, quinoa, and many others, are obviously not included in the ancient wheats category, since they are not part of the genus *Triticum*. This new definition is supported by several authors in literature (Bordes et al., 2008; Ghiselli et al., 2016; Dinelli et al., 2009; Dinu et al., 2018; Valli et al., 2018; Guerrini et al., 2020) and in earlier work (Cappelli et al., 2018; Cappelli et al., 2019a; Cappelli et al., 2020b; Cappelli et al., 2020f; Cappelli et al., 2020g; Cappelli et al., 2020i). Finally, particularly in crisis situations like the actual COVID-19 pandemic, the revival of the cultivation and use of ancient wheats and the strengthening of short food supply chains and local productions, might be essential to take a step forward in the safeguarding of the right of access to healthy and sustainable food (Cappelli et al., 2020b; Recchia et al., 2019).

Despite a more suitable nutritional profile (Dinelli et al., 2009), the ancient wheats are characterized by worse rheological properties of doughs and lower bread volume, compared to doughs and bread obtained by modern cultivars. This highlights the need for technological innovations and improvements, from cradle to grave, for the entire production chain, thus motivating the aims of this thesis. Whole wheat flours, doughs, and bread, are characterized, as the ancient wheats, by higher nutritional content, worse dough rheological properties, and lower bread volumes. This is due to the wheat bran addition which introduces important rheological problems in doughs (Boita et al., 2016), particularly related to the formation of the gluten network.

This negative effect is related to the high concentration of fibers in bran, which are able to hold and bind a large amount of water (Li et al., 2014), leading to a not fully hydrated gluten that has lower tenacity and extensibility (Mastromatteo et al., 2013). More specifically, it is necessary to consider arabinoxylans, inulin, and β-glucans, which are the principal non-starch polysaccharides present in wheat bran. These compounds compete for the usable water with gluten, starch, and the main polymers, leading to an interruption of protein aggregation behavior during heating (Rosell et al., 2010). This behavior, which causes a migration of water from the gluten network to arabinoxylans in whole wheat doughs, has been demonstrated through nuclear magnetic resonance; a significant reduction in water absorption has been found to lead to a deficit in gluten network formation and gas-retention capacity (Li et al., 2014).

A literature review reveals other problems associated with bran addition, such as restricted expansion due to destabilization of the interface between gas bubbles in fermented doughs (Cavella et al., 2008), possible piercing of gas bubbles caused by larger bran particles (Courtin and Delcour, 2002), and opposing opinions regarding the effects of bran particle size reduction on the rheological properties of doughs. The latter has been positively evaluated for an increase of bread volume (Lai et al., 1989), but has been considered negatively by many other authors (Noort et al., 2010), because of an increase in surface interaction and water absorption rate, and also as a result of the liberation of reactive compounds that diminish the aggregation of gluten-forming proteins.

The most noteworthy rheological problems associated with bran addition are an increase in tenacity (P) and viscosity, a decrease in extensibility (L), a decrease in dough strength (W) and finally, a significant rise in the curve configuration ratio (i.e. the ratio between P and L) (Cappelli et al., 2018). Furthermore, the bread produced with whole wheat flour is less performant than refined flours (Zanoletti et al., 2017). In particular, volume is reduced (Tebben et al., 2018) and crumb density and crumb moisture increased (Zanoletti et al., 2017). Consequently, strategies to limit the negative effects of the bran addition and to improve the performance of ancient wheats are indispensable. In chapters 3, 4, 5, 6, and 7, several technological innovations and improvement strategies, with an approach from cradle to grave, were proposed.

1.3 Challenges and opportunities for the flour production chains

As reported in paragraph 1.2, ancient wheats and whole wheat flours production chains need technological innovations and improvement strategies. This needs for ameliorations might lead to the opportunity to improve not only machines, plants, flours characteristics, dough rheological properties, and bread quality, but also the sustainability and the profitability of the entire production chains. In this perspective, the use of ancient wheats, the assessment of the environmental impacts related to bread, pasta, and bakery products productions (through Life-Cycle Assessment (LCA)), and the effectiveness of the substitution of wheat flour with alternative protein sources, such as insects and legumes, might represent innovative and interesting solutions to boost the sustainability of the production chains (Cappelli, 2020).

The environmental impact of wheat production sector can be significant. This has pushed major pasta industries to start evaluating the environmental footprint of their productions by means of Life Cycle Assessment (LCA) and, in some cases, even by Environmental Product Declaration (EPD), according to the standards of the International Organization for Standardization (ISO standards), making information widely available (Recchia et al., 2019). The reasons supporting this choice are mainly due to the increasing attention of final consumers on the impacts of industrial production on the environment, which determine a growing public pressure on this thematic (Fusi et al., 2016; Ruini et al., 2013).

Furthermore, the renewed interest of consumers in ancient wheats has promoted an increase in their cultivation and use, expanding the number of products offered by the baking industry (Cappelli, 2020; Cappelli et al., 2018). This has also contributed to the safeguarding of biodiversity and to the development of a local micro-economy, which allows local producers to increase their profits by differentiating their products (Recchia et al., 2019; Cappelli et al., 2018). The planet protection policy has induced many national and international organizations to support sustainable development, production, and consumption strategies with business support policies. On the contrary, smaller producers usually find difficult to access to this support, especially for niche products, such as pasta and bakery products made following traditional procedures or using ancient wheats varieties.

Bevilacqua et al. (2007), identified the following life cycle phases in the production process of pasta: durum wheat cultivation; milling of durum wheat to obtain semolina; pasta production and packaging; transportation and distribution of final products; domestic consumption, waste and pallet disposal. These phases are also mentioned and quantified in the product category rules and reports of the International EPD ® system for industrial pasta producers. Wheat cultivation is the most variable and, at the same time, fundamental stage. Tillage and all other operations involving soil treatment have the purpose of creating favorable conditions for seed germination and growth, exploiting different techniques. Traditional practices used in Italy are chop residuals of the previous crop, use a moldboard plough to dig the ground at a depth of 0.30 to 0.35 m, and harrow it with one or more passages of a disc harrow. All these operations are carried out as soon as possible after harvesting the previous crop (typically on July, in central Italy).

Sowing of durum wheat is done in mid-autumn with the use of a universal seed drill, and, more rarely, employing pneumatic seeders. The spacing between rows varies from 0.14 to 0.18 m while seed depth varies between 20 and 50 mm. The quantity of seed is approximately 180 to 200 kg per hectare. In durum wheat cultivation performed on a large industrial scale, a minimum tillage is often used with herbicide application before seeding to control weeds. In this case, sowing is done with a direct or combined seed drill, which typically releases up to 220 kg of seed per hectare at a depth of about 50 mm and a 0.2 to 0.3 m row spacing, even if much lower quantities are used in some countries, such as in southern Australia. Fertilizers are applied at variable rates, depending on the soil characteristics: values up to 300 kg/ha of N are quite common in worse conditions. For what concerns the growing period, this occurs from late autumn to late spring all around the world. Watering is usually required in driest areas to achieve satisfactory productivity and quality levels.

Since there are significant differences in durum wheat cultivation, milling methods, and pasta production between high-quality pasta, produced only with ancient wheat varieties in a local or regional scenario, and conventional pasta, produced using national and international wheats following industrial processes, a comparison between the environmental impacts of these two different production chains is necessary to provide specific improvement strategies for the amelioration of both production chains. The results of these comparison, including improvement strategies, opportunities, and future prospects, are reported in paragraph 7.1. Moreover, the LCA tool might be a very powerful instrument for assess, case by case, the environmental impacts of the entire production chain and for propose specific-developed solutions.

Another aspect which might increase the sustainability of the production chain include the substitution of wheat flour with alternative protein sources, such as insects and legumes. Since that the world's population is growing constantly, and is expected to reach 9.7 billion by 2050 (United Nations, 2015), sustainable alternative source of protein need to be found (Cappelli et al., 2020h). This growth goes hand-in-hand with increased demand for protein, exacerbating environmental pressure. There is therefore a need for food production with reduced environmental impact in order to limit Green House Gas (GHG) emissions, reduce energy consumption and optimize land use. All of these factors point to the need for alternative sources of protein (Cappelli et al., 2020h).

Currently, research trends and food innovation are focused on sustainable vegetable and animal protein sources. Among sources of animal protein, insects are a potential solution for the food industry (Cappelli et al., 2020h). Insects are a traditional part of the diet of two billion people, and 1900 species are reported to be regularly consumed (Van Huis et al., 2013). While in Asia, Africa, and South America entomophagy has long been part of the tradition of certain cultures, it has only recently been considered in the Western world. The European Union took a step forward in this direction with the entry into force of Regulation (EU) 2015/2283 (European Parliament and Council of the European Union, 2015) which recognizes insects as a novel food. The use of insects in the food industry might have several positive benefits: firstly, an increase in high-quality protein and nutrients in foods (Rumpold & Schlüter, 2013); secondly, high feed conversion efficiency, which significantly reduces rearing costs (Collavo et al., 2005) and environmental impacts; thirdly, the reproduction rate is higher compared to other animal protein sources (Van Huis et al., 2013); and finally, reduced water consumption and GHG emissions (Oonincx & De Boer, 2012). Neophobia, disgust, and non-acceptance are identified as the major obstacles for the consumption of insects as food in Western countries (Megido et al., 2016). However, studies have demonstrated that food neophobia and disgust decrease if insects are added in an invisible form into products food, such as flour, or if they are associated with known flavors (Megido et al., 2016). As a result, a few innovative studies of insect-based doughs, paste, and products, such as extruded snacks, bread, and meat analogues are reported in literature.

Regarding the safety aspects of insects flours, the microbiological, chemical, physical, and allergenic risks were summarized in earlier work (Cappelli et al., 2020e) and by other authors (Murefu et al., 2019). Despite distinct bacterial communities and different Enterobacteriaceae content between cricket and larvae, Salmonella spp. and Listeria monocytogenes were not detected (Cappelli et al., 2020e). However, it is important to highlight that this does not mean that insects are safe and free from these pathogens (Cappelli et al., 2020e). Another important aspect to be examined is the allergenicity of insects (Cappelli et al., 2020e; Patel et al., 2019). Despite literature shows contradictory results, frying and enzymatic hydrolysis seems to be the most interesting strategies in tropomyosin allergenicity reduction (Cappelli et al., 2020e; Patel et al., 2019).

Legumes are another interesting source of protein as they contain high amounts of essential amino acids such as lysine, threonine, valine, and tryptophan. Cereals, unlike legumes, are rich in sulfur amino acids, and their combination creates a protein with high biological value. Chickpeas, in particular, have high omega 3 fatty acid and lecithin content and can help to control blood pressure, increase HDL cholesterol, and reduce LDL cholesterol (Ranalli et al., 2018). Furthermore, chickpea cultivation reduces the use of nitrogen fertilizers (due to their nitrogen-fixing capacity), with positive impacts for sustainable agriculture and on subsequent crops (Carranca et al., 1999). As a consequence, the literature reports the results of several studies of foods enriched with chickpea flour, such as cakes and bread. In conclusion, in chapter 7, the use of alternative sources of proteins (insects and legume) and the assessment of the environmental impacts of two different pasta production processes were investigated. The results, presented in paragraph 7.1, 7.2.1, and 7.2.2, are in accordance with the recent rediscover interest of consumers in food quality and sustainability.

2 Aims of the thesis

The aim of this thesis is to provide technological innovations and improvement strategies, with an approach "from cradle to grave", to the production chains of flours and bakery products. In order to provide technical solutions and significant ameliorations for farmers, food producers, and food industry, these improvements needs to be focused on the main operations of the production chains (i.e. wheat cultivation, wheat milling, dough processing, and finally, the manufacturing of bread and bakery products). For this reason, the research activities were divided into three main operational phases: Ex-ante, Inter, and Ex-post.

These operational phases embrace the entire production chain, sustaining the approach from cradle to grave. In particular, the Ex-ante phase regard the initial part of the flours production chain. in this phase, the correct management of agronomical treatments, such as nitrogen fertilization, sulfur fertilization, and seeding density, were evaluated with the aim to assess which dosages or combinations might improve flour composition, dough rheology, and bread quality. In particular for ancient wheats, this might be an interesting approach since they usually do not needs significant input of fertilizers and even just a small amount might generate significant improvements in flours and baked products.

The second phase of the research activities, named Inter, is focused on the operations of wheat milling and flour processing to produce bread and bakery products. In particular, the Inter step was aimed to provide improvement strategies and innovative solutions for wheat conditioning, wheat milling (both for stone milling and roller milling), dough rheology, and dough kneading. This phase represent the core business of this thesis. In particular, the strategies for improving wheat milling were examined in dept, both for stone milling and roller milling, with one systematic review of the literature and with two research papers. The same applies to the technological innovations and improvement strategies for dough kneading (one systematic review of the literature and two research papers).

Last but not least, the Ex-post phase. This phase is mainly focused on the increase of the sustainability and in the reduction of the environmental impacts of the entire production chain. This goal is pursued through the assessment of the environmental impact with the LCA analysis and with the use of alternative sources of proteins, such as legumes and insects. Fig. 2 gives a graphical representation of the three main operational phases: Ex-ante, Inter, and Ex-post.

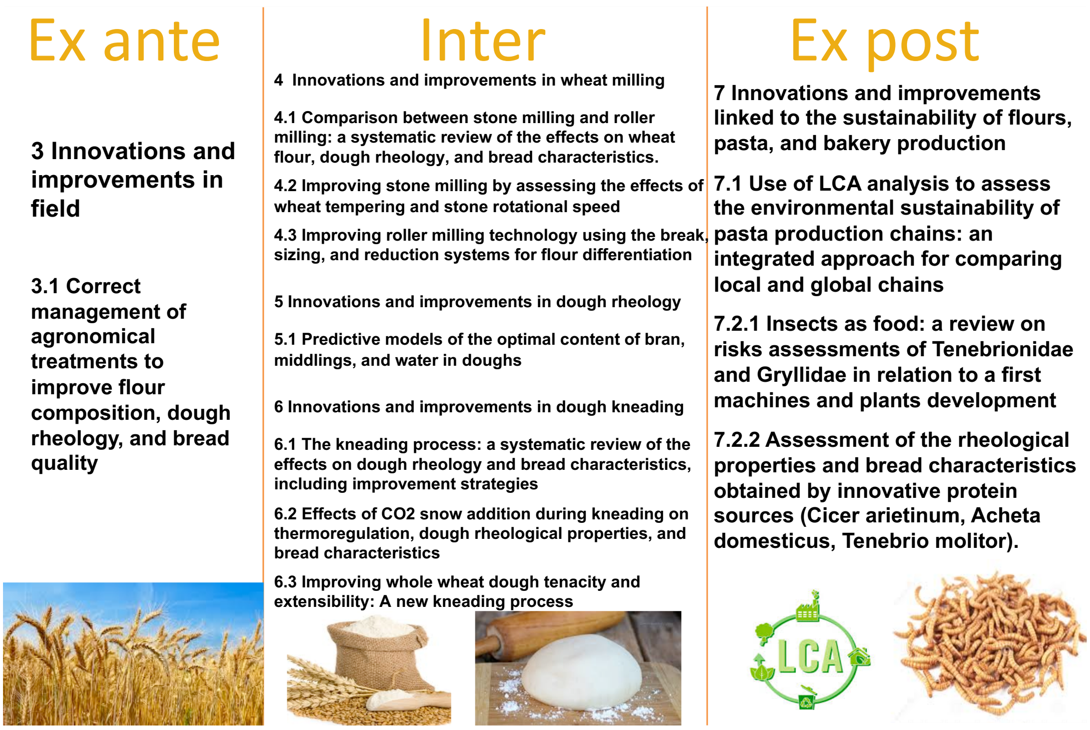

Fig 2: Graphical representation of the three main operational phases: Ex-ante, Inter, and Ex-post.

One of the strategies to reduce environmental impacts of food productions, is the strengthening and the rediscover of short food supply chains and local productions (Cappelli & Cini, 2020d). Particularly during this world crisis due to COVID-19 pandemic, a potential answer to the need to guarantee access to healthy and sustainable food might be furnished by short food supply chains and local productions, which feel less the effect of international restrictions and which, since their rooted presence in the territory, could be closer to the consumers (Cappelli & Cini, 2020d).

For these reasons, is essential to strengthen the research activities to provide technical solutions aimed to improve short food supply chains and local productions, as it is done in this thesis for wheat and flour production chains, because in this crisis (and in potential future menaces even worse), they will represent a potential lifeline (Cappelli & Cini, 2020d). The reinforcement of this local microeconomy is also useful in non-crisis situations, since allow to increase the chances of employment and improve people's quality of life (Cappelli & Cini, 2020d). Sometime, when we are forced to take a step backwards, to have invested in the improvement of short food supply chains and in local productions could let us moving forwards, preserving the access to quality and sustainable food.

3 Ex-ante: innovations and improvements in field

3.1 Correct management of agronomical treatments (nitrogen fertilization, sulfur fertilization and seeding density) to improve flour composition, dough rheology, and bread quality

3.1.1 Aim of the study

Since ancient wheats are less productive and performant than modern wheats, the aim of this research is to assess if agronomical treatments can significantly affect kernel composition, dough rheology and, ultimately, bread quality. Since that sulfur and nitrogen fertilizations could play an important role in final bread quality and given the lack in the literature of works specifically focused on the effects of seeding density, nitrogen fertilization, and sulfur addition on dough rheology and bread quality for ancient wheats, the effects of three agronomical treatments on three ancient wheats varieties, namely Verna, Sieve and Andriolo, were tested.

In particular, thirty-six treatments were investigated. These involved combinations of three varieties of ancient wheats, two seeding densities (90 and 180 kg seed ha−1; D90 and D180 respectively), three nitrogen (N) fertilization levels (35, 80 and 135 kg N ha−1; N35, N80 and N135 respectively), and two sulfur (S) fertilization treatments (0 and 6.4 S kg ha−1; S0 and S1 respectively).

3.1.2 Materials and methods

3.1.2.1 Wheat Cultivation and experimental design

Field experiments were established in October 2016 under rainfed conditions at the Giuseppe Chiarion farm, located in Monteroni d'Arbia, about 20 km south-east of Siena, Tuscany, Italy (43.2007° N, 11.4182° E, 160 m a.s.l.) (Fabbri et al., 2019). Egyptian clover (*Trifolium alexandrinum*, L.) was the previous crop. The soil was silty clay loam, and the 0–0.3 m layer contained 11.4 g kg−1 total organic carbon, 1620 mg kg−1 total nitrogen, 14.2 mg kg−1 available phosphorus, and 273 mg kg−1 potassium. A meteorological station was placed near the experimental field, and data on temperature and humidity were recorded. Three Italian ancient genotypes of wheat (*Triticum aestivum*, L.), namely Andriolo, Sieve and Verna, were assessed.

Thirty-six treatments were investigated. These involved combinations of three varieties of wheat, two seeding densities (90 and 180 kg seed ha−1; D90 and D180 respectively), three nitrogen (N) fertilization levels (35, 80 and 135 kg N ha−1; N35, N80 and N135 respectively), and two sulfur (S) fertilization treatments (0 and 6.4 S kg ha−1; S0 and S1 respectively). The experimental arrangement was a strip-plot design, with wheat cultivars arranged in vertical strips (main plots). N (nitrogen fertilization) was allocated to horizontal subplots, seeding density was applied to vertical sub-subplots, and S (sulfur fertilization) was applied vertically to sub-sub-subplots (Fig. 3).

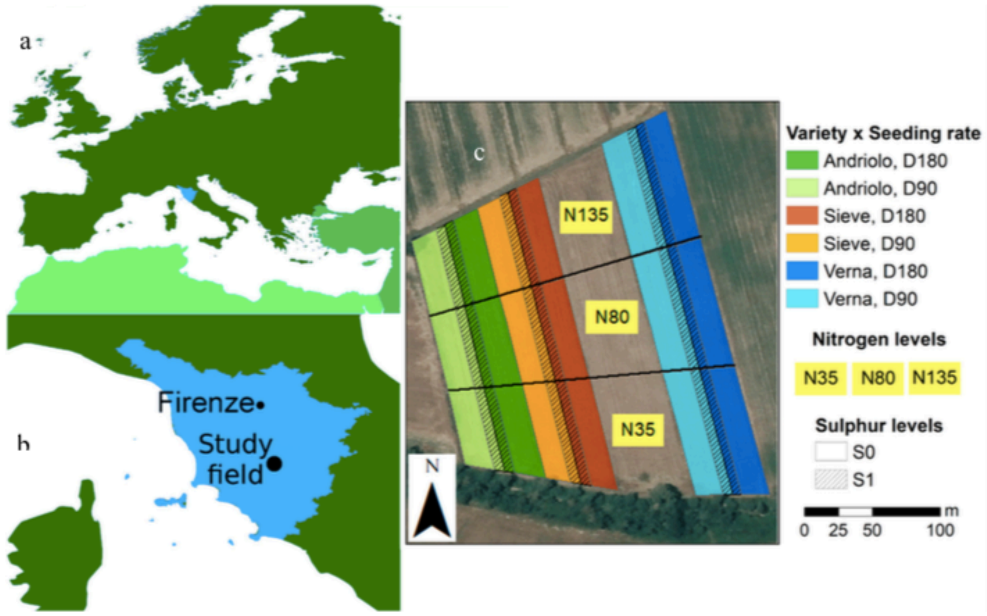

Fig 3: Maps of the study area. (a) Map of Tuscany within Europe. (b) Map of the Chianti region in Tuscany, and the location of the study field. (c) Layout of the study field. Seed densities levels D90 and D180 indicates 90 and 180 kg of seed ha−1, respectively. Nitrogen levels N35, N80 and N135 indicate 35, 80 and 135 kg N ha−1, respectively. Sulfur levels, S0 and S1 indicate 0 and 6.4 kg S ha−1, respectively. The area between the variety Sieve and Verna was cultivated with a 'modern' wheat variety (Triticum aestivum, L. Bologna) with the same cultivation techniques of the ancients wheats (Bologna variety results were not showed in this thesis) (source Guerrini et al., 2020).

Seeds were sown on December 19, 2016. A total of 175 kg ha−1 of triple superphosphate (P2O5: 46%) was broadcast on treatments N35, N80, and N135. Nitrogen total dose was scheduled in three different applications: 20% by broadcasting urea (N: 46%) at seeding, 40% by spreading ammonium nitrate (N: 26%) at tillering, and 40% by spreading urea (N: 46%) at stem elongation.

The S1 treatment was performed at booting by spraying a wettable sulfur powder (Thiovit Jet 80 WG®, Syngenta, Basel, Switzerland) at a rate of 8 kg ha−1 (6.4 kg ha−1 of active ingredient). Although Thiovit is commonly used as a fungicide at a recommended rate of 8 kg ha−1, we tested it as an alternative to sulfur fertilizers. At tillering, an herbicide treatment was performed by distributing Axial Pronto 60 (Syngenta, Basel, Switzerland) at a dose of 0.75 L ha−1 (60 g L−1 Pinoxaden and 15 g L−1 and Cloquintocet-mexyl) and Marox SX (Cheminova Agro Italia, Rome, Italy) at a rate of 0.75 L ha−1 (333 g L−1 of thifensulfuron-methyl and 167 g L−1 of tribenuron-methyl). At booting, a fungicide treatment was performed by spraying Amistar Xtra (Syngenta, Basel, Switzerland) at a rate of 0.8 L ha−1 (Azoxystrobin 18.2% and Cyproconazole 7.3%) and Sakura (Sumitomo Chemical Co., Tokyo, Japan) at a rate of 1.2 L ha−1 (Bromuconazole 167 g L−1 and Tebuconazole pure 107 g L−1).

No noticeable crop damage was observed during the growing season due to weeds, insects, or disease. In particular, no fungal attacks were observed either on surfaces treated with sulfur, or on untreated surfaces. Harvesting was performed at wheat commercial maturity (kernel moisture lower than 13%) on 10 July 2017. The three varieties reached the commercial maturity on the same time. For each treatment, three plant samples were randomly collected from an area measuring 0.5 m2. Wheat from each treatment was harvested separately using a combine-harvester equipped with Trimble GPS sensors, and yield monitoring sensors designed to measure and record information such as kernel flow and moisture, area covered and location. For each treatment, 5 kg of harvested wheat kernel were sampled for quality and technical analyses.

3.1.2.2 Analysis of Kernel

For each kernel sample, the following analyses were performed in triplicate. The hectoliter weight (HW; kg hL^{-1}) and 1000 kernel weight (KW, g 1000^{-1} seeds) were determined according to ISO 7971-1 and ISO 520, respectively. Kernel samples were milled using a grinder with a 0.5 mm screen (Cyclotec 1093 lab mill, FOSS Tecator, Höganäs, Sweden) as reported in Zìlic et al. (2011). Then, wholemeal flour samples (5 mg) were analyzed with a CHNS analyzer (CHN-S Flash E1112, Thermo-Finnigan LLC, San Jose, CA, USA) to determine total nitrogen and total carbon.

3.1.2.3 Analysis of Proteins

A modified Osborne fractionation was performed to isolate the single protein fractions: water soluble (albumins), 0.5 M sodium chloride soluble (globulins), 70% ethanol soluble (gliadins), 0.1 M acetic acid soluble (glutenins), and insoluble proteins. Two hundred grams of raw wheat kernel were milled using the previously described grinder with a 0.5 mm screen (Zìlic et al., 2011). Then, the resulting whole wheat flour (150 g) was defatted using 600 mL of hexane and vortex for 90 min. This suspension was centrifuged (10000 rpm, 10 min) and the extracts discarded. The defatted flour was air-dried under a hood at 20 °C for 24 h. Next, 100 g were sequentially extracted using four solvents (see below) (Guerrini et al., 2020).

Initially, flour was extracted with deionized water (400 mL) for 30 min, vortexing for 1 min, every 10 min. Then, the mixture was centrifuged for 5 min at 2000 rpm. The supernatant was recovered and stored. The extraction was repeated two more times over the resulting pellet with the same solvent; recovered supernatants were combined, designated as the albumin extract, and stored at 4 °C in the dark. The resulting pellet was extracted with 400 mL 0.5 N NaCl solution for 60 min, vortexed for 2 min, at 10 min intervals. Then the mixture was centrifuged at 3000 rpm for 10 min and the supernatant recovered. This extraction was repeated twice and the supernatants were combined (globulin extract) and stored at 4 °C in the dark (Guerrini et al., 2020).

The pellet was then extracted with 400 mL 70% ethanol solution for 60 min, vortexed for 1 min, every 10 min. Then, the mixture was centrifuged for 10 min at 3000 rpm, and the supernatant recovered. The procedure was repeated three times to remove all of the protein in this fraction, before the three supernatants were combined (gliadin extract) and stored at 4 °C in the dark. Finally, the centrifugate was extracted with 400 mL acetic acid 0.1 M for 90 min, vortexed for 1 min, every 10 min and then centrifuged for 5 min at 3000 rpm, to obtain the supernatant (Guerrini et al., 2020).

Acid extraction was repeated three times before the supernatants were combined (glutenin extract) and stored at 4 °C in the dark. Gliadin was precipitated from its extract by adding acetone, following the procedure given in Tecson et al. (1971). Other proteins were precipitated by adjusting pH to 4.1, 4.3 and 4.8, for albumin, globulin and glutenin, following the procedure reported in Ju et al. (2001). The precipitate was oven-dried (105 °C, 5 h) and weighed. Remaining, insoluble protein was determined following the procedure given in Bean et al. (1998).

Solvent residues were removed from the pellet resulting from acid extraction by mixing it with 10 mL of acetone, centrifuging (10000 rpm, 5 min) and discarding the extracts. The pellet was crushed with a mortar and pestle, then oven-dried (105 °C, 5 h). Dried pellets were analyzed to determine total Kjeldahl nitrogen (TKN). The resulting TKN values were converted to insoluble protein by multiplying by 5.7 according to ICC Standard 167 (2000). Total protein was calculated by summing the weight of the five protein groups described above. Protein fractions were expressed as % of total protein on dry weight basis.

3.1.2.4 Analysis of Doughs

The flours obtained by the three ancient wheat cultivars, were sieved to reach the Italian “Tipo 2” law standards, since their ash content ranged from 0.80% to 0.95%. Dough rheology was assessed with the Chopin alveograph according to ISO 27971 procedures (ISO, 2008). Briefly, 250 g of flour was weighted and mixed in the alveograph chamber with a NaCl solution (2.5% w/w) for 8 min (ISO, 2008). Then, dough was extruded and rested for 20 min before the measurement (ISO, 2008). As defined in the standard protocol (ISO, 2008), dough tenacity (P), dough extensibility (L), deformation energy (W), the curve configuration ratio (P/L), and the index of swelling (G) were evaluated.

3.1.2.5 Breadmaking Process

To evaluate the effect of agronomical treatments, breads were prepared using the following recipe: 310 g of flour; 180 g of water; 9 g of NaCl; 12 g of fresh brewer's yeast. Mixing of ingredients (25 min at room temperature), dough formation, resting, leavening (1 h and 20 min at 40 °C) with fresh brewer's yeast (Lievital, Trecasali, Italy), and baking (55 min at 180 °C), were all carried out with a bread machine (Pain dorè, Moulinex, Ecully, France).

3.1.2.6 Analysis of Breads

The standard millet displacement method (Cappelli et al., 2019a) was used to measure bread volume. Specific volume was determined as the ratio between bread total volume and bread weight. Crumb specific volume was determined by cutting 5–10 g of bread crumb and calculating the ratio between its volume measured with the standard millet displacement method and its weight. This is supported by earlier work (Cappelli et al. 2020f) and by other authors (Parenti et al. 2019). Crumb and crust moisture were measured by gravimetry at 105 °C until constant weights were reached.

The Texture Profile Analysis (TPA) of bread samples was carried out by two-bite compression using a Texture Analyzer (TA-XT2, Stable Micro Systems, Godalming, Surrey, England), with a circular flat-plate probe (diameter of 25 mm) according to the procedure described in Kim et al. (2017). Three slices of about 1 cm thickness were cut from the middle of each bread sample. Thus, for each sample, 3 measurement replicates were performed, and the median value was taken. Mechanical test conditions were as follows: 50% compression rate, 50 N of automatic trigger load, 10 mm of travel distance and 3 mm s^{-1} for pre-test, test and post-test speeds. Crumb hardness, cohesiveness, gumminess, chewiness and springiness were measured.

3.1.2.7 Statistical Analysis

A 3-way ANOVA was used to test the main effect of the three agronomical factors and their interactions. Significance was set at $p < 0.05$. When the significance level was reached, a Tukey HSD post-hoc test was run. The inclusion of only one year means that we did not test the varieties, since with different pedo-climatic conditions the varieties response could be different. However, the varieties can still be considered semi-independent replications, which means that the main effects of the agronomical treatments are better defined than if this research had been done on only one variety. The software used was R version 3.6.0.

3.1.3 Results and discussion

The experimental design allowed to evaluate the effect of the three treatments (N fertilization, seed density, and S fertilization). The effect of the three agronomical treatments was evaluated in terms of kernel quality and, particularly, hectoliter weight, 1000 kernel weight, kernel C and N content, total proteins, and protein composition. Average production for Andriolo, Sieve, and Verna varieties (Table 1) was 2925, 4204, and 2955 kg ha−1, respectively.

Table 1: Mean of kernel quality parameter results by nitrogen fertilization, sulfur fertilization, seed density, cultivar and first order interaction. The sig columns report the ANOVA results (= 0.05, ** = 0.01, *** = 0.001, ns = not significant), while lowercase letters represent the Tukey HSD post hoc test results. RSE = residual standard error (Source: Guerrini et al., 2020).*

Source of Variation	Kernel Yield ($kg\ ha^{-1}$)		1000-Kernel Weight (g)		Hectoliter Weight ($kg\ hL^{-1}$)		Total Nitrogen (%)		Total Carbon (%)	
	Average	*sig*	Average	*sig*	Average	*sig*	Average	*sig*	Average	*sig*
Main effect										
Cultivar										
Andriolo	2925.43		44.32		71.83		2.21		45.8	
Sieve	4203.85		40.89		74.05		2.2		45.51	
Verna	2955.07		37.46		72.35		2.28		45.46	
Seeding density		ns		**		ns		ns		ns
D90	3395.04	a	41.84	a	72.94	a	2.20	a	45.57	a
D180	3327.86	a	39.94	b	72.54	a	2.26	a	45.61	a
Sulfur		ns		ns		ns		ns		*
S0	3357.21	a	41.51	a	72.58	a	2.16	a	45.85	a
S1	3366.16	a	40.27	a	72.89	a	2.3	a	45.33	b
Nitrogen		**		ns		ns		**		ns
N35	2667.66	b	41.16	a	72.98	a	2.07	b	45.45	a
N80	3107.08	b	40.81	a	72.12	a	2.12	b	45.71	a
N135	4309.6	a	40.7	a	73.12	a	2.49	a	45.61	a
RSE	564.24		1.84		1.89		0.24		0.36	
Interactions										
Nitrogen × Sulfur		ns		ns		ns		*		ns
Nitrogen × Seed Density		ns		ns		ns		ns		ns
Sulfur × Seed Density		ns		ns		ns		ns		ns

Kernel yield was significantly and positively related with nitrogen fertilization. However, differences were found to be related to N135, while no significant differences were found for N35 and N80. On the contrary, seeding density and sulfur treatment resulted not significantly affect final kernel yield. Results indicated 1000 kernel weight significantly decreasing as the seeding density increase from 90 to 180 kg seed ha−1. No difference (except those related to cultivars) was found for hectoliter weight. Kernel N was significantly increased by N135, while no significant differences were found for the other fertilization levels. Moreover, results also indicated a significant positive interaction between S and N fertilization in increasing the N accumulation in kernel. Kernel C content was found to be significantly decreased by the sulfur treatment (from 45.82% ± 0.24% to 45.33% ± 0.50% from S0 to S1). However, the decrease in C could not be considered important in terms of kernel quality.

These results were consistent with previous studies (Gooding et al., 2002; Zhang et al., 2016; Salvagiotti et al., 2009), reporting nitrogen fertilization increasing the kernel yield. Further, other studies reported a positive interaction between N and S fertilization in increasing kernel yield in wheat. Otteson et al. (2008), found that

N concentration in kernel being significantly increased by N fertilization, while being not significantly affected by seeding rate. Gooding et al. (2002) and Zhang et al. (2016) found a significant interaction between N fertilization and seeding density in determining the kernel yield. A significant increase in kernel total protein content was due to nitrogen fertilization (Table 2).

Table 2. Mean of kernel protein fraction results by nitrogen fertilization, sulfur fertilization, seed density, cultivar and first order interaction. The sig columns report the ANOVA results (= 0.05, ** = 0.01, *** = 0.001, ns = not significant), while lowercase letters represent the Tukey HSD post hoc test results. RSE = residual standard error. (source: Guerrini et al., 2020).*

Source of Variation	Total Protein (%DW)		Insoluble Proteins (%DW)		Albumins (%DW)		Globuilins (%DW)		Gliadins (%DW)		Glutenins (%DW)		Total Gluten (%DW)	
	Average	*sig*	Average	*sig*	Average	*sig*	Average	*sig*	Average	*sig*	Average	*sig*	Average	*sig*
Main effect														
Cultivar														
Andriolo	12.41		0.35		1.97		1.13		3.92		5.04		8.96	
Sieve	12.37		0.36		1.92		1.10		3.86		5.13		8.99	
Verna	12.84		0.37		1.98		1.16		4.05		5.28		9.33	
Seeding density		ns		ns		ns		ns		ns		ns		ns
D90	12.18	a	0.35	a	1.94	a	1.12	a	3.90	a	5.05	a	8.95	a
D180	12.67	a	0.37	a	1.97	a	1.14	a	3.96	a	5.28	a	9.24	a
Sulfur		ns		ns		**		**		*		*		**
S0	12.21	a	0.35	a	2.05	a	1.23	a	4.3	a	4.27	b	8.57	b
S1	12.86	a	0.36	a	1.86	b	1.02	b	3.55	b	6.07	a	9.62	a
Nitrogen		**		*		*		ns		ns		ns		*
N35	11.7	b	0.34	b	1.81	b	1.04	a	3.68	a	4.83	a	8.51	b
N80	11.98	b	0.35	b	1.85	ab	1.08	a	3.72	a	4.99	a	8.71	ab
N135	13.93	a	0.4	a	2.2	a	1.28	a	4.38	a	5.68	a	10.06	a
RSE	1.34		0.03		0.24		0.31		0.57		0.63		0.40	
Interactions														
Nitrogen × Sulfur		*		ns		ns		ns		ns		ns		ns
Nitrogen × Seed Density		ns		ns		ns		ns		ns		ns		ns
Sulfur × Seed Density		ns		ns		ns		ns		ns		ns		ns

Moreover, significant differences were found between N135 level and the two lower levels, while no significant difference was found between N35 and N80. On the contrary, the adopted nitrogen fertilization levels had little effect on the protein composition of these old wheat varieties. Nitrogen fertilization significantly affected albumin content, which increased slightly (from 1.81% to 2.2% of DW), but only for the N135 treatment. Further, N fertilization significantly affected total gluten, which increased by about 18.2% from N35 to N135.

In contrast, modern wheat varieties have been reported to be more sensitive to the amount of N, expressed in both their yield potential and protein composition, while it appears to have little effect on albumins and globulins content (Fuertes-Mendizábal et al., 2010; Pedersen & Jørgensen, 2007). It has also been reported to positively influence total gliadins and glutenins. Results also indicated that the sulfur treatment deeply changed the protein composition. In particular, the sulfur treatment significantly decreased albumin, globulin and gliadin fractions, while it significantly increased glutenin (from 4.23% to 6.07% of DW). Moreover, also total gluten significantly increased by about 12.3% from S0 to S1. Tao et al. (2018) reported that sulfur availability was positively correlated with glutenin production and negatively correlated with the ratio of gliadin to glutenin. To sum up the results of the impact of our factors on kernel proteins, nitrogen fertilization increased total protein and total gluten content, while the sulfur treatment changed the protein composition, increasing total gluten and glutenins, and decreasing other protein fractions.

Agronomical treatments affected dough rheology and, particularly, dough tenacity (P), dough extensibility (L), and deformation energy (W) (Table 3). On the other hand, the index of swelling (G) showed no significant difference. P increased slightly with sulfur and nitrogen fertilization. Dough extensibility was increased by nitrogen fertilization. Since dough deformation energy is the area under the tenacity and extensibility curve, both an increase in P, and an increase in L increase W. In fact, W increased by about 34% with nitrogen fertilization (from N35 to N135), compared to roughly 14% with the sulfur treatment. Increases due to the agronomical treatment are of particular interest for old wheat flours, especially when processed as Italian “Tipo

2” flours, as they are considered usually very weak in term of deformation energy, and any increase in this value has to be considered helpful for the breadmaking process (Ghiselli et al., 2016).

Table 3. Mean of alveograph results by nitrogen fertilization, sulfur fertilization, seed density, cultivar and first order interaction. The sig columns report the ANOVA results (= 0.05, ** = 0.01, *** = 0.001, ns = not significant), while lowercase letters represent the Tukey HSD post hoc test results. RSE = residual standard error. (source: Guerrini et al., 2020).*

Source of Variation	W		P		L		P/L		G	
	Average	*sig*	Average	*sig*	Average	*sig*	Average	*Sig*	Average	*Sig*
Main effect										
Cultivar		***		***		ns		***		ns
Andriolo	52	c	33	c	44	a	0.85	c	14.5	a
Sieve	94	a	57	a	40	a	1.53	a	13.9	a
Verna	60	b	40	b	40	a	1.06	b	13.0	a
Seeding density		ns		*		ns		ns		ns
D90	72	a	45	a	43	a	1.12	a	14.6	a
D180	65	a	42	b	39	a	1.17	a	13.7	a
Sulfur		*		*		ns		ns		ns
S0	64	b	42	b	39	a	1.14	a	13.7	a
S1	73	a	45	a	44	a	1.15	a	14.5	a
Nitrogen		**		*		*		ns		ns
N35	58	b	41	b	36	b	1.21	a	13.2	a
N80	69	ab	44	ab	41	ab	1.1	a	14.2	a
N135	78	a	45	a	46	a	1.13	a	14.9	a
RSE	11		4		10		0.29		1.7	
Interactions										
Nitrogen × Sulfur		ns		ns		ns		ns		
Nitrogen × Seed Density		ns		*		ns		ns		
Sulfur × Seed Density		ns		ns		ns		ns		

Nitrogen fertilization increased the Verna W from 48 to 67 × 10−4 J, the Andriolo W from 49 to 54 × 10−4 J and the Sieve W from 78 to 111 × 10−4 J. These values are consistent with alveograph evaluations in Migliorini et al. (2016) for Verna and Sieve, while they are lower for Andriolo. Overall, W values remain low regardless of the agronomical treatment and, in fact, according to the common classification, flours with W below 90 are not considered suitable for breadmaking. In our study, Sieve, at nitrogen levels N80 and N135, exceeds this threshold and can be considered a weak flour. In this case, nitrogen fertilization was able to change the flour ‘class’.

W values were compared to the literature with respect to total protein content (Migliorini et al., 2016) and their high molecular weight glutenin content. Our data are consistent with earlier work, as both W and total proteins increased with nitrogen fertilization. Furthermore, a significant relationship between W and total protein was found (p = 0.008). Although we only have only data for total glutenin, a significant relationship between glutenin content and W was found (p = 0.02). In this case, both W and glutenin were found to be significantly affected by the S treatment. This is consistent with an effect of S fertilization on dough technological parameters reported in Tea et al. (2005). On the other hand, neither nitrogen nor sulfur were able to change the P/L ratio. High P/L is another limit of ancient wheat flours, but our data did not highlight any change due to the agronomical treatment.

Our agronomical treatments did affect final bread quality (Table 4). Crumb density was significantly decreased by nitrogen fertilization. Crumb density is an important parameter for bread quality, since it can be considered a proxy for bread porosity. Nitrogen fertilization leads to higher protein content in kernel, higher W and, consequently, higher crumb density. Furthermore, nitrogen fertilization also affected crumb texture. Crumb springiness and crumb cohesiveness increased with nitrogen fertilization from N35 to N135 (by 17% and 32% respectively). Springiness describes how the crumb returns to its un-deformed state after a compression force is removed, while cohesiveness describes the amount of effort required to chew, and it is usually seen as a positive characteristic in baked products.

Nitrogen fertilization improved both these parameters, resulting in an improvement in bread crumb texture. The tested agronomical treatments, nitrogen and sulfur, significantly increased the total protein content of Italian “Tipo 2” flours; in particular, an enhancement of the storage proteins (i.e., gluten), was obtained. The effect of the agronomic treatments on the gluten proteins was different: nitrogen treatment equally increased glutenin and gliadin fractions, maintaining their ratio roughly unvaried; conversely, sulfur reduced the gliadins and enhanced the glutenins, determining a change in the proportion of the two components of the gluten. In the literature it is largely known that gluten plays a key role for the flour breadmaking performance, since it confers the dough unique viscoelastic properties. Hence, sulfur and nitrogen treatments, impacting the gluten quantity, revealed that an agronomic practice could directly affect the most important actor in the breadmaking process.

This observation was well known for modern refined varieties, and could be extended to Italian “Tipo 2” flours from old varieties. Rheological results showed a significant boost in the dough strength (W) as a consequence of both sulfur and nitrogen treatments. These results could be related to the higher gluten quantity of Italian “Tipo 2” flours, since in the literature it is largely known that gluten proteins significantly improve dough rheological/alveograph properties. W represents an important parameter in the evaluation of the flour technological quality: the higher the W index, the higher the dough stability during mixing, gas holding capacity and performance during long fermentation time, since the alveographic test simulates the deformations occurring during the leavening and baking steps (Cauvain et al., 2004). Furthermore, in the literature, W values are commonly used to classify flours for their destination use (Cauvain et al., 2004).

With regard to the evaluation of bread quality, nitrogen treatment produced a significant decrease of bread crumb density, and a significant increase of texture parameters, namely springiness and cohesiveness. These results were consistent with data about the gluten content and alveographic parameters. Indeed, the increased gluten proteins and W value allowed a better gas retention capacity of the dough during the leavening and the baking. During leavening, the gluten promotes a better retention of the gas produced during the yeast fermentation, allowing a better loaf increase; during baking, it allows the creation of a fine-even crumb while water evolves as vapor and gases further expand (Cauvain et al., 2004).

As a result, bread crumb appeared characterized by a significant lower density and by a porous structure. Moreover, TPA analysis showed that this crumb structure was characterized by a significant increase of springiness and cohesiveness. Both these texture parameters are associated to a better bread quality and are features largely appreciated by consumers. Conversely, sulfur fertilization, although producing similar effects of nitrogen in term of W, did not significantly affect bread characteristics. This is probably linked to the observed decrease in the gliadin fraction (Barak et al., 2013).The sulfur results highlight the importance of the ratio between gliadin and glutenins to obtain a bread with quality characteristics appreciated by consumers. Furthermore, it is important to point out that all the cited literature referred to breads made from refined flours, while these results allow to evaluate the effect of agronomical treatments on bread quality. Moreover, both chemical and rheological tests showed improvements in the Italian “Tipo 2” flour composition and dough rheology that not resulted in a significant improvement of the bread. Thus, the nitrogen fertilization could be useful to improve the poor technological features of weak flours.

Table 4. Mean of quality analyses on breads shown by nitrogen fertilization, sulfur fertilization, seed density, cultivar and first order interaction. The sig columns report the ANOVA results (= 0.05, ** = 0.01, *** = 0.001, ns = not significant), while lowercase letters represent the Tukey HSD post hoc test results. RSE = residual standard error. (Source: Guerrini et al., 2020).*

Source of Variation	Volume (mL)		Crumb Density (g mL^{-1})		Hardness (N)		Springiness (mm)		Cohesiveness		Chewiness (N·mm)	
	Average	*sig*	Average	*sig*	Average	*sig*	Average	*sig*	Average	*sig*	Average	*sig*
Main effect												
Cultivar		***		ns		ns		ns		ns		ns
Andriolo	1043	b	1.42	a	11.7	a	0.74	a	0.32	a	2.57	a
Sieve	1165	a	1.24	a	7.3	a	0.81	a	0.41	a	2.44	a
Verna	993	c	1.36	a	8.6	a	0.75	a	0.39	a	2.34	a
Seeding density		ns		ns		ns		ns		ns		ns
D90	1052	a	1.26	a	8.3	a	0.80	a	0.39	a	2.51	a
D180	1082	a	1.42	a	10.2	a	0.74	a	0.36	a	2.39	a
Sulfur		ns		ns		ns		ns		ns		ns
S0	1079	a	1.31	a	10.2	a	0.78	a	0.39	a	2.35	a
S1	1054	a	1.37	a	8.3	a	0.76	a	0.37	a	2.55	a
Nitrogen		ns		*		ns		*		*		ns
N35	1039	a	1.50	a	11.4	a	0.70	a	0.31	a	2.29	a
N80	1077	a	1.29	b	7.4	a	0.79	b	0.41	b	2.21	a
N135	1085	a	1.23	b	8.9	a	0.82	b	0.41	b	2.85	a
RSE	57		0.26		4.7		0.10		0.09		0.95	
Interactions												
Nitrogen × Sulfur		ns		ns		ns		ns		ns		ns
Nitrogen × Seed Density		ns		ns		ns		ns		ns		ns
Sulfur × Seed Density		ns		ns		ns		ns		ns		ns

3.1.4 Conclusions

The aim of the study was to evaluate whether the poor technological bread-making qualities of three ancient wheats flours could be improved with different agronomical treatments. Thus, we tested the effect of nitrogen fertilization, sulfur fertilization, and seed density on kernel composition, dough rheology, and bread quality.

Results related to seed density were minor and cannot be used to improve the breadmaking properties of the tested varieties. Sulfur fertilization was found to affect protein composition and, particularly, increase gluten content. W values consistently increased with sulfur addition. Since W is a key parameter in the assessment of flour workability, a sulfur foliar application in such weak flour could be a promising strategy to improve their technological performance. However, further studies on a broad range of varieties, with in-depth chemical analyses are still required to fully understand the effect.

Finally, nitrogen fertilization was found to be a useful tool to modulate the assessed qualitative parameters as it was able to increase yield, total protein, total gluten content, and protein composition. Furthermore, nitrogen fertilization improved the W value of the dough, and changed bread crumb density and texture. Hence, N fertilization can be successfully used to improve technological parameters of the tested weak flours.

In conclusion, the poor performance of these flours can be improved with agronomical treatments designed to obtain higher-quality bread. These results can be considered of particular interest for ancient wheats with poor technological performance. However, more work is needed in order to make further improvements to their processability. Moreover, additional trials including more years and different pedo-climatic conditions are required to evaluate the interaction between cultivars and the agronomical treatments.

4 Inter: innovations and improvements in wheat milling

4.1 Comparison between stone milling and roller milling: a systematic review of the effects on wheat flour quality, dough rheology, and bread characteristics

4.1.1 Aim of the study

Grain milling might be the oldest manufacturing process in the world. Archaeologists have found drawings, dating back to 2600 BC, of the basic process of rubbing or grinding using two stones on the walls of Egyptian tombs (Walker & Eustace, 2016). Grinding (milling) of cereal grains is the fundamental operation currently used to produce flour, which is an essential ingredient in many food products (Liu et al., 2018). Although nowadays there are many milling techniques that use mechanical force to break grain into smaller fragments or fine particles (Liu et al., 2018), stone mill and roller mill continue to dominate in the food industry (Doblado-Maldonado et al., 2012; Liu et al., 2018).

Selecting the optimal milling process is a key consideration in production, because the physicochemical and functional properties of wheat flour, dough, and bread are significantly affected by different methods (Doblado-Maldonado et al., 2012; Li et al., 2014). Many studies have shown that when different cereal grains (e.g. wheat, rice, sorghum, barley, rye) are milled, the selected milling method affects the color, particle size, surface area, bulk density, damaged starch, and the structure and functional properties of the flour, leading to flours with different physicochemical properties (Albergamo et al., 2018; Cubadda et al., 2009; Ficco et al., 2016; Kihlberg et al., 2004; Liu et al., 2018; Palpacelli et al., 2007; Yu et al., 2018). Yu et al. (2018) noted that these differences are mainly due to milling conditions, notably mechanical forces, intensity, etc., and that the milling process is an important factor in determining the quality, performance, and suitability of end products.

Since that the food industry is continuously seeking strategies and techniques that can improve milling operations, the first aim of this research is to summarize current knowledge regarding the effects of stone mill and roller mill on wheat flour quality, dough rheology, and bread characteristics. The second aim is to suggest specific strategies to improve stone milling and roller milling by increasing efficacy, efficiency, and the quality of the final product, with positive impacts on production, profitability, and environment.

4.1.2 Search strategy

The literature review used two search strings (one for roller mill and one for stone mill) to explore three databases: ScienceDirect, PubMed, and the Web of Science. The search strings used were as follows:

- Wheat AND ("stone milling" OR "stone grinding")
- Wheat AND ("roller milling" OR "roller grinding")

No language, time, or publication status restrictions were imposed, and duplicates were excluded. The initial results were screened by reading the title and abstract (articles that only consisted of an abstract and/or index were excluded at this point), and then by a full text reading. All articles concerning any aspect of SM or RM of cereals were included, while those that were not relevant to their effects on flour quality, dough rheology, or bread characteristics were discarded. For each type of milling, a flow chart was produced to summarize the obtained results (Fig. 4).

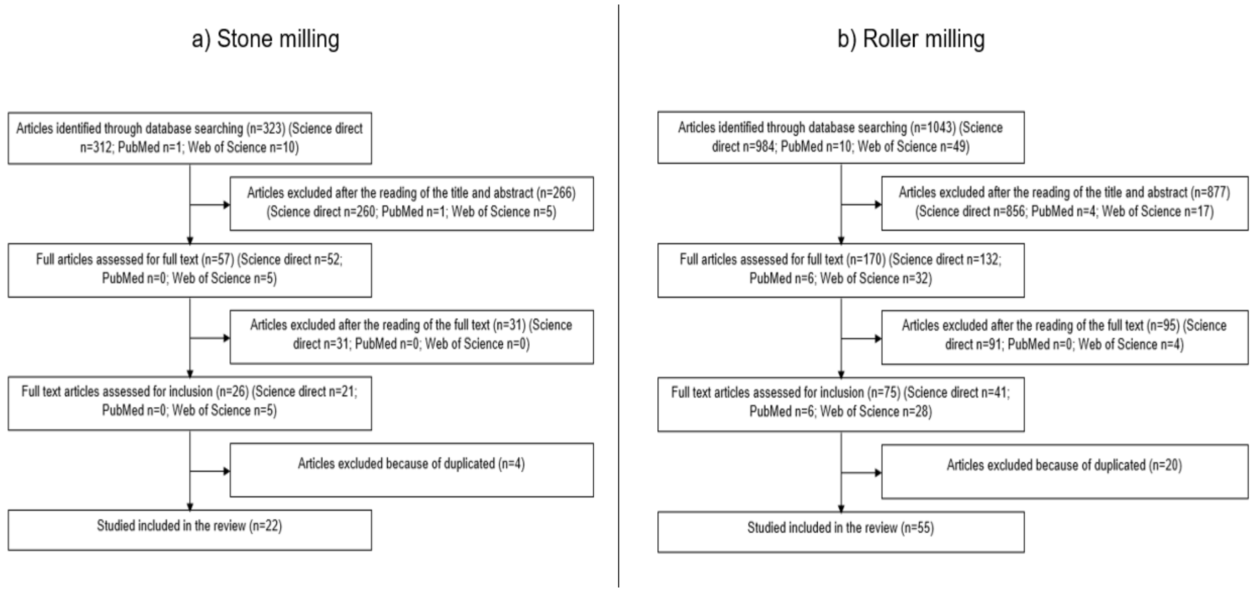

Fig 4: Flow charts pertaining to the selection of papers on stone milling (a) and roller milling (b), obtained according to the results of the systematic literature review. (Source: Cappelli et al., 2020i)

4.1.3 Wheat milling

Nowadays, the two most widely-used wheat processing techniques in the food industry are stone mill and roller mill (Doblado-Maldonado et al., 2012; Liu et al., 2018). The following paragraphs describe their functioning, effects on wheat flour, dough, and bread, and improvement strategies.

4.1.3.1 Stone milling

Stone mills are the oldest form of attrition mill used to produce flour. They simultaneously use several physical forces, such as shear, compression, and abrasion, to mill wheat kernels between two stones, with a theoretical extraction rate of 100% (Kihlberg et al., 2004). Contemporary systems use composite millstones attached to metal plates (Doblado-Maldonado et al., 2012). Wheat is crushed between the two stones, and the output is a whole wheat flour containing all kernel fractions (endosperm, bran, middlings, and germ). Stone mill is very simple and quick, and is the easiest way to produce whole wheat flour (Zhang et al., 2018). If the aim is to produce whole wheat flour, the process ends here. If, however, the aim is to produce refined white flour, the output is sifted in centrifugal sifters (in small and medium-sized plants) or in plansifters (in larger plants). The centrifugal sifter consists of a rotating cylindrical sieve and a counter-rotating internal paddle that throws the product against the sieve, forcing the fine fraction through, while the coarse fraction tails over (Posner, 2003). At the end of the process, three distinct fractions are collected: refined white flour, bran, and middlings.

The literature notes that stone mill generates considerable heat due to friction and other physical forces, which can result in considerable damage to starch, protein, and unsaturated fatty acids, compared to other milling techniques (Prabhasankar & Rao, 2001). The latter study found slight protein degradation in samples milled at 55 and 85 °C using stone and plate mills. However, data regarding amounts of nutrients such as protein and fat were not supported by appropriate statistical analyses, and no information was given on other nutritional/ nutraceutical properties. Nevertheless, it is clear that heat is a process parameter that should be controlled and kept as low as possible. Interestingly, there appears to be a marketing advantage in using the term “stone ground” with consumers, as evidenced by the prevalence of whole wheat flour products in both retail and commercial markets. The main benefit of stone mill is that the endosperm, bran, and germ fractions remain in their natural proportions in whole wheat flour, which is perceived by the consumer as a “natural” product. Vendors can guarantee that all of the valuable nutritional/nutraceutical compounds are present, as most phytochemicals are concentrated in the germ and the outer bran layer of the wheat kernel (Di Silvestro et al., 2014). The stone mill claim has become so important to consumers that some mills have started to “crack” the grain using a stone mill, before the cracked wheat is reduced to flour via roller mill (Doblado-Maldonado et al., 2012).

4.1.3.2 Roller milling

Roller milling involves the separation of the endosperm from the bran and germ, followed by a gradual reduction in endosperm particle size (Campbell, 2007). Wheat passes through a series of rollers (rolls), accompanied by sifting between stages (Doblado-Maldonado et al., 2012). Rolls may be smooth or fluted, according to the stage of the process. Corrugations on fluted rolls do not run parallel to the long axis, but instead are arranged in a spiral pattern to obtain a scissor action. Fluting has either a steep (sharp) or shallow (dull) profile, and rolls can be arranged in a dull-to-dull, sharp-to-sharp, dull-to-sharp, or sharp-to-dull sequence (Rosentrater & Evers, 2017). This sequence can significantly impact particle size distribution of the flour (Fang & Campbell, 2003).

Rolls are driven by flat belts. Their speeds are controlled by a cogwheel or chain drive. Chains or stepped gears enable the distance between axes of pairs of rollers to be adjusted, without changing the transmission wheels. The material to be ground is fed into the machine by two feed rolls (Meuser, 2003). Wheat is ground between two rolls that rotate against each other and are positioned horizontally. Commercial rolls range from 180 to 350 mm in diameter and can be up to 1500 mm in length. In terms of performance, smaller-diameter rollers are more suited to shearing actions that aim to separate endosperm and bran. On the other hand, larger-diameter rolls create a longer grinding path that acts on the material mainly through compression. Most modern roller mill are equipped with rollers that have the same length and diameter, in order to achieve maximum uniformity and avoid maintaining a large inventory of spare rollers (Posner, 2003).

Roller mill is characterized by high pressures that, like stone mill, inevitably lead to increases in temperature. In roller mill, the problem is managed by passing cooling water through the hollow center of the rollers. Another issue arising from the continuous, high-pressure process is adhesion of the processed material to the roller surface, which can block the flutes of corrugated rolls and create an irregular increase in the effective diameter of smooth rollers. Surface adhesions can be removed by scrapers mounted below smooth rollers; unfortunately, they are not appropriate for corrugated rollers, where strong-bristled brushes are employed (Rosentrater & Evers, 2017).

Roller mill efficiency depends on the amount of material fed into the rollers, the type of corrugation, and their differential speed. The amount of material that is processed is limited by the air cushion formed over the rollers, which is, therefore, frequently reduced by means of feed mechanisms that aim to increase efficiency (i.e. by drawing off the air above the gap and compacting the material to be ground). The differential speed can be as high as 1:15, depending on the flour product. At this ratio, the fixed, driven roller is reduced to the circumferential speed of the movable roller. The upper limit of the circumferential speed is around 6 ms^{-1}.

Typically, corrugated and smooth rollers with a standard diameter have a circumferential speed of 3.3–4.4 ms^{-1} during wheat and rye milling (at 250–360 rpm) (Meuser, 2003).

Roller mill is characterized by four distinct phases (Sakhare & Inamdar, 2014):

I. The break system

The first stage is known as the break, and the aim is to separate the endosperm from the bran and germ (Rosentrater & Evers, 2017). The idea is to open the kernel and scrape the endosperm off the bran; this is when most of the endosperm separation is achieved (Campbell et al., 2007). Rosentrater and Evers (2017) noted that although some flour is produced, that is not the purpose of this stage. Instead, the intention is to produce large fragments of endosperm that are fed into the reduction system and purifiers. The system consists of four break rolls that are typically followed by sieving (although this step may be omitted when an eight roller mill system is used). The flutes on the first break rolls are the largest (3.5–1 cm). They shear open the wheat kernel, ideally along the crease, and unroll the bran coat to form an irregular, relatively thick layer of endosperm that is pressed onto a thin sheet of bran. Overtails are conveyed to the second break roller mill, in which the rolls are less coarsely fluted (5.5–1 cm), and the gap between rolls is narrower. The largest particles from the following sieving stage pass to the third break, where they are ground by finer-fluted rolls with a narrower gap, and so on to the fourth and, potentially, a fifth break. The details of the system vary significantly among mills, and the applicable wheat type is determined by the number of break stages and grist.

II. The sizing system

Next, smooth, sizing, and reduction rollers are employed to reduce the endosperm to fine flour (Posner, 2003). In some milling systems, the largest endosperm particles may be ground by finely fluted rollers, called sizing rollers. The purpose is to scrape bran particles from chunks of endosperm, so that the two components can be easily separated by purifiers, before further endosperm reduction (Rosentrater & Evers, 2017).

III. The reduction system

The reduction system consists of 6–12 milling stages, interspersed with sifting that removes the flour produced by the preceding grind (Rosentrater & Evers, 2017). Milling is carried out with rollers that differ in two significant ways compared to break rollers: their surface is smooth or, more often, slightly matt; and the speed differential between them is lower (usually 1.25:1), although the fast roll still runs at 500–550 rpm. The purpose of the system is to crush and shear the grain, while the balance between the two forces is controlled by the smoothness of the rolls' surface (Rosentrater & Evers, 2017).

IV. The tailings system

Heterogeneous materials left over from each stage are sent to a sieving machine, called a plansifter (Posner, 2003). This is an enclosed unit that contains up to 10 stacks (compartments) of up to 32 sieve frames. The exact number of frames varies according to the sieved material and the manufacturer. Sieves are arranged in groups that differ in terms of their aperture size. Sieve aperture (measured in microns) is established by the miller. The mill designer assigns a certain surface area to each sieving stack. Overtails are directed to the next stage in the process for further milling or purification (Posner, 2003).

4.1.4 Main findings

4.1.4.1 Results of the systematic literature review

A total of 1366 initial items were obtained. Following the application of the selection criteria described in section 4.1.2 (removal of duplicates and screening), 77 texts were selected. Duplicates that had been included twice, because they referred to both roller mill and stone mill, were also removed, leaving a final total of 66 items: 11 book chapters, 6 reviews, and 49 research papers. 19 of these were published in the last five years. Fig. 4 summarizes, in the form of flow charts, the selection process, which is consistent with the PRISMA statement (Moher et al., 2009). Fig. 4 (a) and (b) show the results for stone mill and roller mill, respectively.

4.1.4.2 Effects of stone milling on wheat flour, dough, and bread

For consumers, millers, and bakers, one of the most important aspects of wheat flour quality is its nutritional profile. Recently, interest has grown in the use of stone mill, due to the widespread opinion that stone-milled flours have a better nutritional profile than roller-milled flours. The marketing advantage is evidenced by the recent preponderance of stone milled products in both retail and commercial markets. Stone mill has been found to have very little effect on macroelement losses (sodium, magnesium, potassium, calcium, and phosphorus) and no effect on microelement losses (manganese, iron, copper, zinc, and selenium), reinforcing the idea that it produces flours with high nutritional value (Albergamo et al., 2018). Moreover, trace element loss has been confirmed to be much less severe than in conventional roller mill. Cubadda et al. (2003) found average losses of iron, copper, zinc, and selenium ranging from 33 to 81% in white durum wheat flour. These findings are consistent with those by Ficco et al. (2016), who showed that stone mill preserves more compounds with high value for health, such as total fibre, carotenoids, and, in the purple wheat genotype, anthocyanins.

Albergamo et al. (2018) reported that stone mill, like roller mill, cannot reduce mycotoxins. However, the latter paper contradicts a comparative study reported by Palpacelli et al. (2007), who showed that stone mill reduces the vomitoxin and zearalenone content in flour, unlike roller mill. Specifically, the mean vomitoxin content in stone milled flours was found to be 170 ppb, compared to 360 ppb for roller milled flours, while the zearalenone content showed a similar difference (6 ppb vs 13 ppb). These results were confirmed by the analysis of commercial flours, which found that vomitoxin in stone milled flours was 245 ppb, compared to 945 ppb in roller milled flours, and the zearalenone content was 1.7 ppb and 6.0 ppb, respectively. Stone mill therefore appeared to result in a reduction of about 40–50% in the vomitoxin and zearalenone contents. The authors argued that these results were related to the stone mill trimming machine they used. This machine was equipped with an aspirator that was able to, at least partially, eliminate the outermost layer of the wheat kernel, where most of the mycotoxins are located. Furthermore, their roller mill system used a series of reduction rollers to extract residual flour from the bran, which, the authors argued, was likely to increase mycotoxin extraction.

Crude fat content has been found to be significantly higher in stone milled flours (2.1%) than roller milled flours (0.9%) (Liu et al., 2018). The same study also found a significant difference for protein content; amounts were highest in stone milled flours (11.4%), and significantly lower for roller milled flours (7.3%). Stone milled flours contained higher amounts of crude fibre, ash, potassium, calcium, magnesium, iron, manganese, copper, and zinc than roller milled flours. However, Liu et al. (2018) emphasized that these results were obtained at low stone rotational speed, which limits heating. Regarding phenolic compounds, the same study found that stone milled flours contained higher amounts of protocatechuic acid, vanillic acid, epicatechin, ferulic acid, rutin, and ellagic acid, compared to roller milled flours. This finding was confirmed by total phenolic content, total flavonoids content, and antioxidant activity, which were significantly higher for stone milled flours. Di Silvestro et al. (2014) observed that heat stress, caused by stone mill, might lead to an increase in amylose content. Lin and Czuchajowska (1996) noted that this damage should be considered during baking, as damaged starch must be minimized in wheat flour used to make sourdough bread, to ensure adequate gassing during fermentation, while excessive damage can lead to a sticky dough and an undesirable red crust color. Finally, Liu et al. (2018) emphasized that the moisture content of stone milled flours (12.7%) was significantly higher than in roller milled flours (12.0%), and that it had higher water holding capacity.

Regarding the effects of stone mill on wheat flour characteristics, Liu et al. (2018) found that stone mill produced flour with a narrower particle size distribution and more uniform particle size, compared to roller mill, while Kihlberg et al. (2004) found that stone milled flour had a wider particle size distribution in the central range (> 400 – ≤ 800 μ), compared to roller milled flour. Bayram and Öner (2005) emphasized that the particle surface of stone milled bulgur was smoother and the shape was more regular, due to the abrasion effect. Stone mill smooths the surface and improves the appearance of the particles; in particular, they are not split or glassy, and their surface is opaque. Furthermore, in the case of coarse bulgur (Pilaf) obtained from stone mill, the yield was highest (83.1%). Bayram and Öner (2005) concluded that, although alternative milling techniques and systems for bulgur processing were needed, stone mill still seemed to be the best current method.

With respect to dough, Kihlberg et al. (2004) found that doughs made with whole wheat stone milled flour had a more homogenous particle size than those made with roller milled flour. This was because the majority of the stone milled flour fractions had the smallest size distribution, and the endosperm was separated from the bran to a lesser degree. Regarding rheological properties, Kihlberg et al. (2004) found higher values of falling number for whole wheat stone milled flour compared to roller milled flour, while stone mill samples showed lower water absorption compared to roller mill samples. The opposite trend was observed for conventional wheat milling. Image analyses of bread showed that the milling technique was also able to influence loaf volume (measured as slice area) and had the greatest impact on baking performance, measured as the area of the middle slice of the loaf, which was larger for stone mill than roller mill samples in whole wheat bread. The slice area of breads baked with stone milled flour was larger than for breads baked with roller milled flour. Furthermore, when the bran particle size was smaller, and still partly connected with endosperm, as in stone milled flour, deformity increased.

Kihlberg et al. (2004) also found that the milling technique had a significant impact on the sensory qualities of whole wheat flour products. Their study showed that whole wheat breads baked with roller milled flour and stone milled flour differed in their sensory attributes, due to differences in the physical processing of the wheat. Whole wheat bread produced with stone milled flour was characterized by the crispiness of the crust, its roasted cereal aroma, saltiness, and crumb deformity. Crust crispiness may have been related to the heat produced during flour milling. Furthermore, the starch in the dough that formed the crust of the bread baked with stone milled flour was less damaged and more directly exposed to heat, despite the standardized baking process used. Stone mill influenced not only the crust, but also the crumb, thereby affecting both texture and flavor, with consequences for the saltiness attribute. This was not unexpected, as the bran contains a rich combination of compounds. Phenolic compounds are mostly derivatives of benzoic acid, and include ferulic acid, resorcinol, aromatic amines, phytates, folates, and sterols. At the same time, the smaller size of the bran particles in stone milled flour facilitated reactivity, thus affecting the intensity of taste perceptions.

4.1.4.3 Effects of roller milling on wheat flour, dough, and bread

Regarding wheat flour characteristics, Kihlberg et al. (2004) reported that roller milled flour had more damaged starch than did stone milled flour. Liu et al. (2018) found that total starch content was significantly higher in roller milled flour (77%) compared to stone milled flour (70%). Roller mill produced flour that had a wider particle size distribution than stone mill (Kihlberg et al., 2004; Liu et al., 2018). Notably, distributions were widest in the ranges > 1120 μ and ≤ 250 μ (Kihlberg et al., 2004). Roller mill had negative effects on the mineral content of flour and semolina (Cubadda et al., 2009). The latter study found that roller mill had the most influence on the mineral content of semolina. At least six groups could be distinguished: selenium had the highest decrease (77–85%) (dry weight basis), followed by calcium (54–60%), copper (49–53%), potassium and phosphorous (42–47%), iron (36–38%), magnesium and zinc (32–36%). Furthermore, in roller mill, wheat germ was subject to more pronounced oxidation and rancidity phenomena (Boukid et al., 2018).

With respect to dough rheological properties, the falling number for whole wheat roller milled flour was found to be lower compared to stone milled flours (Kihlberg et al., 2004), and juiciness was reported to be greater in breads produced with roller milled flours. Furthermore, the latter authors found that breads made with roller milled flour had higher maximum consistency, water absorption, dough development time, and stability. Roller mill also affected bread characteristics: the image analysis described in Kihlberg et al. (2004) showed that the milling technique had a great impact on loaf volume, measured as the area of the middle slice of the loaf, in whole wheat breads. Volumes were larger for whole wheat breads made with stone milled flour compared to roller milled flour. Dough rheology and the baking performances of roller milled flour could be improved with a slight extension of kneading time, while this was not the case for stone milled flour. Cappelli et al. (2019a) proposed another strategy to improve whole wheat dough and bread. As the roller mill process separates white flour, bran, and middlings, the authors suggested delaying the addition of the bran and middlings during kneading. They found that a delay of 2 min (25% of the total kneading time) improved the dough rheology (reduced tenacity and tenacity/extensibility ratio, and increased extensibility) and bread characteristics (greater specific volume).

Kihlberg et al. (2004) found that wheat flour characteristics and specifically the amount of damaged starch produced by milling were enough to influence the sensory qualities of whole wheat bread, and to distinguish breads obtained from roller milled flour and stone milled flour. Furthermore, the impact was greater than that of the farming system or of the amount of flour in the formulation. In fact, their study found that the milling technique had the greatest impact on baking performance. The sensory attributes of whole wheat breads differed significantly, due to the mechanical and physical processing of the wheat. Sweetness, juiciness, compactness, and the raw streak of the crumb were the main sensory attributes noted for the whole wheat bread produced with roller milled flour, and samples were generally characterized by a higher intensity of wheat aroma.

4.1.5 *Strategies for improving stone milling*

4.1.5.1 Keep the milling temperature as low as possible and avoid heating

Prabhasankar and Rao (2001) found that the key disadvantage of stone mill was that it generated considerable heat (due to friction), which damaged starch, protein, and unsaturated fatty acids. Their study found a slight degradation in protein, in samples milled at 55 and 85 °C using stone and plate mills. However, data regarding the effects on other nutrients were not supported by appropriate statistical analyses, and no information was given about other nutraceutical components. Nevertheless, it is clearly important to keep temperatures low during stone milling, in order to avoid heating that could lead to nutrient loss. This observation is supported by the findings described above, which highlighted the fact that stone mill has less severe effects on macronutrients and micronutrients than does roller mill (at low temperatures and low stone rotational speed). Given that stone mill maintains the natural proportions of bran, middlings, and germ in whole wheat flour, and the consequent marketing advantages (Di Silvestro et al., 2014), systems need to be developed that can cool down the stones used in stone mill, following the model of existing systems used for cooling in roller mill.

4.1.5.2 Correct management of wheat feed rate and the distance between stones

Ghodke et al. (2009) emphasized the importance of correct regulation in stone mill. The authors tested feed rates of 0.21, 0.63, and 1.05 min for 200 g of wheat grain, with apertures (distance between the stones) of 2, 3, and 4 mm. Increasing the feed rate and aperture decreased the damaged starch content in the resulting flour, and vice versa. Aperture had the largest effect on damaged starch formation, while dough stickiness was dependent on feed rate. Highest stickiness was found at the slowest feed rate. Tear was lowest and moisture content was highest with the smallest aperture. The smaller aperture resulted in increased damage to flour starch and, hence, increased water absorption and softening of the dough. The study showed that feed rate and aperture were important process parameters that needed to be correctly set as a function of the desired flour characteristics.

4.1.5.3 Wheat conditioning

Wheat conditioning (also known as tempering) consists of the addition and absorption of water by the kernel (Posner, 2003). The aim is to ensure that the pericarp and germ are tough and plastic, and do not splinter during milling. At the same time, the endosperm should be as friable as possible, in order to optimize milling. Various studies have investigated conditioning, which has been found to have five purposes: (i) to toughen the bran, thus reducing the formation of bran powder and contamination (Boukid et al., 2018; Dexter & Sarkar, 2004; El-Porai, et al., 2013; Kweon et al., 2014; Posner, 2003); (ii) to soften the endosperm, thereby enhancing its milling ability (Boukid et al., 2018; Dexter & Sarkar, 2004; El-Porai et al., 2013; Kweon et al., 2014; Posner, 2003); (iii) to facilitate the separation of bran from endosperm, reducing evaporative losses (Dexter & Sarkar, 2004; El-Porai et al., 2013); (iv) to ensure easy and accurate sifting of stocks (El-Porai et al., 2013); and (v) to ensure that endosperm moisture content is sufficient to result in a final moisture content of flour of between 14 and 15% (El-Porai et al., 2013; Kweon et al., 2014). However, the amount of water to be added and the correct tempering time vary widely, and there is no one regime that is universally appropriate for all wheat types and milling systems. Typically, soft wheat is conditioned to 15–15.5% (Kweon et al., 2014) and hard wheat to 16–16.5% (Posner, 2003).

Conditioning time is very important. El-Porai et al. (2013) studied three times (12, 24, and 36 h). The results showed that 12 h increased flour yield, 24 h increased dough tenacity and extensibility, and 36 h increased dough deformation energy. Although mill type and speed have greater effects on dough rheological properties and bread characteristics, conditioning time seems to be an interesting avenue for further investigation. Tempering time, temperature, and final moisture content should be carefully considered, as these three variables affect the milling process (Posner, 2003). Achieving optimum wheat moisture content before milling is critical (Dexter & Sarkar, 2004). The latter authors found that too much tempering reduces flour yield, because it becomes more difficult to completely separate bran from endosperm, and sieving efficiency is reduced. Too little tempering results in bran powdering, which contaminates the flour. Nozzles are used to spray water onto the wheat as it moves along an enclosed screw conveyor, and modern systems include an electronic unit that controls the precise and uniform addition of moisture.

Regarding energy consumption, Warechowska et al. (2016) and Dziki (2008) highlighted the fact that conditioning significantly increased the specific grinding energy and the grinding efficiency index. Furthermore, increasing moisture content significantly increased the average size of ground wheat particles, notably the bran fraction (Warechowska et al., 2016). The latter authors found that flour yield decreased by 12–18% regardless of genotype, and ash, protein, and gluten content decreased as moisture increased. The decreased ash content was considered to be an improvement in flour quality. An opposite trend was observed for the gluten index and water absorption; here, increased moistening contributed to the mechanical strengthening of the gluten network, thus enhancing sorption capacity. Finally, an increase in water absorption and better stability of the dough was noted for Astoria and Cytra cultivars.

Dziki (2008) proposed an interesting strategy to reduce the specific grinding energy, based on wheat crushing before milling. The paper suggested that crushing considerably decreased grinding energy requirements, for both soft and hard cultivars, with a reduction in average particle size distribution of ground material (in particular, an increase in the fraction < 200 μm) in soft wheat. Cappelli et al. (2020f), assessed the effects of wheat conditioning on refined white flour quality, dough rheological properties, and bread characteristics. The authors highlighted the fact that wheat conditioning at 13% increased the farinograph dough stability, which successively decreased as the conditioning moisture increased (i.e. 15 and 17%) (Cappelli et al., 2020f; Cappelli et al., 2020i). Furthermore, regarding Chopin alveograph results, Cappelli et al. (2020f and 2020i) reported that the dough tenacity (P) and the curve configuration ratio (P/L) decreased as the conditioning moisture increased, for the softening of the doughs. An inverse trend was observed for dough extensibility (L), which instead, increased with the increase in conditioning moisture (Cappelli et al., 2020f; Cappelli et al., 2020i). Finally, regarding the results of wheat conditioning on bread characteristics, Cappelli et al. (2020f and 2020i) highlighted that the higher bread specific volumes and the lower crumb densities were obtained with a wheat conditioning of 15% and 17%. Despite the latter article examined in depth the topic, further studies are necessary. In conclusion, the correct management of tempering could significantly affect the milling process, and could improve dough rheological properties and bread characteristics.

4.1.5.4 Rediscover and modernize traditional stone watermills

The traditional watermill, described by Aristotle in 400 BC, uses water fed through a gear mechanism (Walker & Eustace, 2016; Cappelli et al., 2020i). Di Silvestro et al. (2014) emphasized the fact that stone milled flour obtained using a traditional watermill (at temperatures of around 30 °C) is different in several ways, compared to modern stone mill flour (obtained at 60 °C). Their study found that lower total starch was obtained using a modern stone mill (61.6 g) compared to a watermill (68.1 g), and the higher temperature led to an average loss of 6.5 g of starch per 100 g of flour. Although flours obtained with the modern stone mill had higher amounts of insoluble dietary fibre, watermill stone milled flours contained more polyphenols and flavonoids, making the latter technique a very interesting way to increase nutraceutical content. Not only does the lower milling temperature have positive effects on flour quality, but traditional stone watermills could have other advantages: lower environmental impacts (due to the use of green energy); improvements to the landscape and attractiveness of rural areas; opportunities to produce flour with higher polyphenols; and a potential marketing advantage related to the promotion of tradition and craftsmanship (Cappelli et al., 2020i).

4.1.6 *Strategies for improving roller milling*

Both the literature and the evolution in milling techniques confirm the advantages of roller mill compared to stone mill. The first advantage relates to better efficiency and flexibility. As reported by Doblado-Maldonado et al. (2012), efficiency was improved by narrowing the gap and using smooth, rather than corrugated, rollers during reduction. Flexibility is another important advantage. The diameter, surface structure, rotational and differential speeds of rollers can all be configured (Meuser, 2003). Moreover, milling and reduction can be varied for each roller and for different raw materials (Doblado-Maldonado et al., 2012). This wide range of adjustable operating parameters is not possible in stone mill. The second advantage is lower heat generation compared to stone mill (and the ability to install cooling systems). The last (but not least) advantage is linked to the ability to separate bran and middlings. Unlike stone mill, which can only output whole wheat flour, roller mill can separate the bran, middlings, and germ from the endosperm fraction for further processing (Chun-feng et al., 2006; Gili et al., 2017; Srivastava et al., 2006). These strategies can result in inhibited lipase activity, which is concentrated in the bran, thus extending the shelf life of whole wheat flours (through enzyme inhibition), without influencing the flour's functional properties (Doblado-Maldonado et al., 2012).

Wheat conditioning is an improvement strategy that is also applicable to RM. Strategies specific to roller mill are described in the following subsections.

4.1.6.1 Wheat debranning combined with the processing of bran, middlings, and germ

Nowadays, many industrial mills include debranning in aestivum and durum milling, as it increases capacity, enhances yield (De Brier et al., 2015), and improves semolina quality (Bottega et al., 2009; Rosentrater & Evers, 2017). Debranning removes a portion of the bran before the first break, through the application of friction and abrasion forces, without nicking the endosperm, but which limit the number of broken kernels. This facilitates the separation of the germ after the first break, thus enhancing flour quality (Bottega et al., 2009), although this strategy seems to be most suited to *Triticum durum* (Dexter & Wood, 1996). It also appears to increase refinement, and the nutritional content of by-products that hold great promise as novel food ingredients (Dexter & Wood, 1996). Moreover, the process improves milling performance and can simplify flows in mill plants (Dexter & Wood, 1996).

Lijuan et al. (2007) reported that debranning had several positive aspects, such as a decrease in the gluten index, reduced starch damage, optimal falling number, and increase in the maximum resistance of dough. In addition, debranned flour was whiter than non-debranned flour and had higher peak viscosity. Furthermore, Dexter and Wood (1996) reported increases in deformation energy, flour yield, pasta brightness, and mineral, vitamin, and polyphenol content in debranned fractions. Positive effects were observed on bread quality scores, such as volume, vol/wt, height, and structure of steamed bread, increased ash and pericarp content, and particle size distribution (Lijuan et al., 2007). Debranning was an effective way to obtain wheat bran fractions that were enriched in phenolic compounds and antioxidants, with reduced α-amylase activity (debranning from 3 to 6% of kernel weight) (De Brier et al., 2015). This could increase the health benefits associated with whole wheat products (Beta et al., 2005). However, debranning has very different effects on soft and hard wheats, and further studies are needed to understand the performance of doughs made from flours obtained using different milling and debranning methods.

Debranning is even more interesting in the context of whole wheat flour production and storage. Studies have found that significant enzyme inactivation is achieved if the debranned fraction is stabilized, using light steam treatments (Srivastava et al., 2006), microwaves (Chun-feng et al., 2006), or infrared radiation (Gili et al., 2017), before its reinsertion into the refined flour, thus increasing whole wheat flour storage time and improving the nutritional characteristics of whole wheat bread. Infrared radiation is another heating method for bran processing. That method reduces processing time, lowers energy consumption, only requires simple equipment, and has no toxicological or environmental effects. It also reduces phytic acid content, but does not affect the content of crude fat, protein, and phenolic compounds. Another interesting strategy to stabilize bran, middlings, and germ involves the use of microwaves. Microwave heating at 900 W for 120 s has been found to decrease lipase activity and the moisture content of the wheat bran, thus extending its storage period (Zhang et al., 2018). Lipase activity is the first step in lipid degradation, and enzyme activity is concentrated in the bran; consequently, this fraction can be treated separately, before being added back to refined wheat flour to make whole wheat flour. This method extends the shelf life of whole wheat flours (by the inhibition of enzymes), without influencing their functional properties (Doblado-Maldonado et al., 2012).

Furthermore, laboratory and industrial debranning techniques have proved effective in reducing the mycotoxin content of wheat, although their efficiency is extremely variable; for example, the reduction of deoxynivalenol in debranned wheat was found to range from 15 to 78% (Cheli et al., 2013). Rosentrater and Evers (2017) and Posner (2003) highlighted several other advantages of debranning: a reduction in bacterial, fungal, and other contamination associated with the outer layers of the kernel that are removed in the early stages of the milling process. Zhang et al. (2018) and Rosentrater and Evers (2017) noted a reduction in the enzyme activity concentrated in the external bran layers. The aleurone layer can be reintroduced, after being processed and stabilized, into the resulting flours, thus improving their nutritional value (Rosentrater & Evers, 2017). The roller mill process can be shortened for grains that have been decorticated – coarsely fluted rolls used in the conventional first break are not required and can be substituted with sizing rolls (Posner, 2003). Finally, extraction rates for flour and semolina are higher, while bran contamination may be reduced, when grains are decorticated before milling (Posner, 2003; Rosentrater & Evers, 2017).

4.1.6.2 Cooling down the rollers

Rollers are often called ‘chills’, as they can be cooled (Posner, 2003; Rosentrater & Evers, 2017). Fast cooling after casting ensures that the carbon is mixed with steel, creating a hard roller surface, while slow cooling allows the carbon present in the steel to crystallize, resulting in a softer surface (Posner, 2003). Rollers can heat up considerably during grinding, due to friction; this makes it difficult to maintain the aperture, particularly when the rollers are subject to a high degree of wear. To remedy this problem, hollow, water-cooled rollers have been developed – rollers are cooled by pumping water over the shaft. This has been found to improve milling performance, leading to a more uniform result (Meuser, 2003).

4.1.6.3 Correct setting of differential ratios

Posner (2003) studied the differential ratio between rollers, which ranged from 1.2:1 to 1.5:1. Lower differentials have a larger compression effect on the wheat kernel. Smooth rollers are placed closer together, in order to reduce endosperm chunks in the flour and, as a result, require considerably more power than corrugated rollers. The miller must control compression to avoid damage to the flour and prevent excessive flaking of endosperm particles. Flaked endosperm can reduce flour yield, because overtails from the top sieves during reduction ends up in the mill's overtails streams and, subsequently, the bran stream (stock that has gone over a sieve is known as ‘overs’).

4.1.6.4 Development and improvement of automatic and adaptive mill plants

From a long-term perspective, in particular regarding industrial-scale roller mill, the development and use of Programmable Logic Controllers and Programmable Automation units will make it possible to set, on a case-by-case basis, the correct amount of water provided during conditioning, the distance between rollers, differential ratios, wheat feed rate and other fundamental milling parameters that can reduce consumption and waste, and enhance final flour product quality. These systems could be based on data-driven systems, such as model-based predictive controls (Dal-Pastro et al., 2017; Owens, 2001), compositional breakage equations and functions (Galindez-Najera et al., 2016; Mateos-Salvador et al., 2013), and the discrete element method (Patwa et al., 2016). The aim is to develop an intelligent grain processing system that is able to automatically manage milling by, for example, the optimal selection of processing conditions, the early detection of faults in real-time, and real-time product quality prediction (Dal-Pastro et al., 2017; Owens, 2001). Although further investigations are necessary, we are not so far from making all this possible.

4.1.6.5 Purification of by-products

Purification is one of the three major steps in the roller mill process (Posner, 2003). A purifier consists of a long, oscillating sieve with a slight descending slope (different according to the types), which is divided into four or more sections. The material to be purified becomes progressively coarser from the head to the tail. Air currents are drawn through one to three layered sieves that move simultaneously with alternating motion. Individually controlled air currents rise through the cover, aspirating the stock as it moves over the oscillating sieve cover, and heavier particles tend to fall through. Purification relies on materials of different sizes, density, and air resistance (Posner, 2003; Rosentrater & Evers, 2017). Controlled air currents separate heavier particles (relatively pure endosperm), which flow through the sieves, and lighter ones (with higher bran content), which tail over the sieves (Posner, 2003). This process of stratification results in, from top to bottom: light bran, heavy bran, large composites, large pure endosperm particles, and small pure endosperm particles (Rosentrater & Evers, 2017). Relatively pure endosperm particles are sent to reduction rollers to increase flour production, while overtails is directed to sizing rollers or tail-end fine breaks. Grinding, sifting, separation, and regrinding are repeated, until the endosperm is reduced to an acceptable degree, and, in particular, no more flour can be separated from the bran. Each flour stream differs in quality, due to its origin within the wheat kernel, equipment adjustment, stage of the milling process, etc. The collected flours are mixed, and then transferred by screw conveyors to storage bins (Posner, 2003).

4.1.7 Conclusions & future trends

This work has highlighted the importance of selecting the optimal milling technique, as a function of the desired flour quality, dough rheology, and bread characteristics (Cappelli et al., 2020i; Albergamo et al., 2018; Cubadda et al., 2009; Ficco et al., 2016; Kihlberg et al., 2004; Liu et al., 2018; Palpacelli et al., 2007; Yu et al., 2018). Only a few studies have specifically compared stone mill and roller mill techniques, which have pointed up the need for extensive comparative studies of their different effects. Nevertheless, this review has revealed several important observations regarding the effects of the two milling techniques (Cappelli et al., 2020i).

The benefits of stone mill are: its simplicity (Zhang et al., 2018); the higher concentration of macroelements, microelements, and polyphenols in flours milled at low temperature and at low stone rotational speed (Albergamo et al., 2018; Cubadda et al., 2003; Ficco et al., 2016; Liu et al., 2018); and a marketing advantage (Di Silvestro et al., 2014). Other, potentially positive effects are its ability to reduce mycotoxins (Palpacelli et al., 2007), the higher protein and fibre contents of the flour (Liu et al., 2018), and higher whole wheat bread volume (Kihlberg et al., 2004). However, these results need to be validated by further comparative studies. On the other hand, it is difficult to manage milling parameters, and there are other negative effects, notably, heat generation (Prabhasankar & Rao, 2001), not optimal falling number, and poorer dough rheological properties (Kihlberg et al., 2004), indicating that there is significant room for technological improvement.

On the other hand, roller mill also has clear advantages in terms of efficiency and flexibility (Doblado-Maldonado et al., 2012; Meuser, 2003). Positive effects include lower heat generation, optimal falling number, and better dough rheological performance (Kihlberg et al., 2004; Posner, 2003). Although this technique has already been significantly improved at the technological level, much can still be done to improve performance. Section 4.1.6 highlighted the fact that particular attention should be paid to wheat debranning and the stabilization of bran, middlings, and germ (a combined strategy that is not yet widely applied). The application of this solution could lead to enzyme and microorganism inactivation, the stabilization and extension of whole wheat flour shelf life, and, for millers, a simplified strategy to manage whole wheat flour storage and orders (Cappelli et al., 2020i).

This review has not declared a “winner”, because both stone mill and roller mill techniques have advantages and disadvantages (Cappelli et al., 2020i). The optimal milling system is a function of the aim of the process, keeping in mind business needs and flour product demand. For example, stone mill is the best option if the aim is to produce flour with high nutritional content (macroelements, microelements, polyphenols, and fibre) and market appeal (Cappelli et al., 2020i). On the other hand, roller mill is best if the miller has to provide large quantities of refined white flour with more preferred dough rheological properties and higher kneading resistance (Cappelli et al., 2020i). To support these different aims, many modern mills are equipped with both stone mill and roller mill. Finally, it is important to emphasize that the milling technique is only one of many factors that affect flour quality, dough rheological properties, and bread characteristics. Agronomic practices, the selection of the right cultivar, and optimal wheat moisture content are just some of the other parameters that need to be considered. A final consideration is the choice of improvement strategies for both stone mill and roller mill, which should also be based on the goal of the milling process (Cappelli et al., 2020i). The rational and optimized development of innovations and improvements will have positive effects, not only on flour product quality, but also on the productivity and profitability of the entire production/supply chain (Cappelli et al., 2020i).

4.2 *Improving stone milling by assessing the effects of wheat tempering and stone rotational speed on particle size, dough rheology, and bread characteristics*

4.2.1 *Aim of the study*

Milling (or grinding), is an essential stage in the processing of wheat grains, which are used to produce wheat flours. The two predominant techniques are stone and roller mills (Cappelli et al., 2020i; Doblado-Maldonado et al., 2012). Although stone milling is an old technology, it is still preferred by some consumers (Cappelli et al., 2020f). Although the literature shows that stone mills can generate considerable heat, the ability to label a product as made with "stone-ground flour" is a powerful marketing tool for both producers and retailers (Cappelli et al., 2020i; Cappelli et al., 2020f). With respect to roller mills, several factors can be controlled that influence milling performance and flour quality, with a consequent effect on dough rheological properties and bread characteristics (Cappelli et al., 2020i). In contrast, fewer adjustments can be made in stone milling; notably optimizing wheat moisture and stone rotational speed seem to be the most interesting strategies to improve the end product.

Wheat moisture is controlled via a process known as conditioning or tempering (Cappelli et al., 2020f). Water is added to the wheat before milling, and it is important to wait until the water has penetrated to the core of the kernel (El-Porai et al., 2013). The aim is to facilitate the separation of bran from endosperm. In fact, on the one hand, the operation reduces bran friability while, on the other hand, it enhances starchy endosperm fragility (Pagani et al., 2014). A few theoretical studies have investigated this topic (El-Porai et al., 2013; Warechowska et al., 2016), notably describing how tempering affects the roller mill process. However, to the best of the authors' knowledge, tempering has not been studied in the context of stone milling (Cappelli et al., 2020f). Moreover, it is possible that in this context there are additional benefits, as the lack of adjustable parameters makes process control more difficult, increasing the variability of the produced flour. Tempering may help to standardize the wheat entering the mill and, consequently, could be a way to reduce flour variability.

Stone rotational speed is another important factor that influences milling performance and flour quality (Cappelli et al., 2020i; Cappelli et al., 2020f). The rotational speed of the mill affects flour quality, particle size distribution, milling time, and specific energy. Improving control of the milling process appears to be particularly important for weak wheat varieties, where all of the potential of the grain must be exploited. Recently, several ancient, weak wheat varieties have been discovered to have specific nutritional and environmental benefits (Dinelli et al., 2009; Recchia et al., 2019). Verna, in particular, has been found to have several beneficial effects on human health (i.e. improvement of lipidic and hemorheological parameters) (Dinelli et al., 2009). However, flour weakness and the addition of bran and middlings produces a dough characterized by low strength and a high tenacity/extensibility ratio (Cappelli et al., 2018), which is difficult to transform into bread. The resulting breads usually have low specific volume and crumb porosity, as well as high crumb hardness (Cappelli et al., 2019a). Finally, these grains, which are only grown in specific regions, are often highly variable as a result of seasonal trends. This high variability makes baking even more difficult. Hence, the aim of this study is to assess whether wheat tempering and the milling speed can improve the poor technological characteristics of a weak flour.

4.2.2 Materials and methods

4.2.2.1 Raw materials and wheat tempering

Investigations were carried out on wheat (Verna) kindly provided by Bocciolini Silvano farm (Florence, Italy). Grain was purified before conditioning, which was performed in tightly-sealed plastic bowls. Moisture content was set at 11%, 13%, 15%, and 17% through the addition of the correct amount of water via a spray atomizer. For each moisture content, three samples of 3 kg were prepared and milled at one of three stone rotational speeds (173, 260, and 346 rpm, corresponding to 18.1, 27.2, and 36.2 rad/s respectively). This procedure was performed in three replicates. Tempered samples were mixed and left to stand for 24 h before milling, to permit the penetration of water to the core of the kernel (Warechowska et al., 2016). Then, a 10 g sample was extracted to verify that the moisture content was correct, using gravimetry at 105 °C until a constant weight was reached. Fresh brewer's yeast (Zeus, Zeus IBA Ltd., Florence, Italy), salt (Chantesel Ltd.), and water (Sant'Anna, Fonti di Vinadio Ltd.) were purchased in a local supermarket. Ash (AOAC 923.03 (AOAC International, 2005)) content of white flour was determined by the Analytical Food Laboratory (Florence, Italy) following approved, official methods.

4.2.2.2 Milling and energy consumption

Milling was carried out with a stone mill (M400, I.B. Manufatti Ltd., Appignano, Macerata, Italy). The stone diameter was 0.4 m. Refined white flour, bran, and middlings were sieved at 180 µm, 500 µm, and 950 µm, respectively, in order to obtain the refined white flour (Type 00 according to Italian legislation – ash content 0.48 g/100 g flour). The three stone rotational speeds were measured using a contact tachometer (DT-2236, Lutron Electronic Enterprise) and several operative parameters were measured or calculated. Milling time was measured with a chronometer (accuracy ± 0.1 s), while the weight of grain before milling and obtained flour fractions were measured to an accuracy of 0.1 g. After milling, total yield was determined as the ratio of the weight of the obtained whole flour and the initial weight of conditioned grain. Whole wheat flour was sifted to separate refined white flour from bran and middlings, and determine the respective yields. Productivity (whole and white) was calculated as the ratio of flour and time. Specific energy consumption (SE) during tests was measured with an ammeter (Lafayette ltd.), and calculated as follows:

$$SE = \frac{V\,A\,t}{cos\varnothing\;\mu\;W}$$

where V is voltage, A is amperage, t is time, µ is milling yield and W is grain weight.

For whole flour, SE was calculated using total yield, while for white flours it used white flour yield. Flour temperature was measured with a thermal imaging camera (E60bx, FLIR Systems Inc.) immediately after milling. Refined white flour was stored in paper bags for one month at 15 °C. Particle size of obtained flours was determined with a laser particle size analyzer (Mastersizer 3000, Malvern, UK).

4.2.2.3 Experimental design

This work assesses differences in flour yield, flour particle size, milling efficiency, dough rheology, and bread characteristics as a function of two factors: wheat moisture and stone rotational speed. With respect to tempering, four moisture levels were tested: 11%, 13%, 15%, and 17%. Regarding stone rotational speed, three levels were tested: 173, 260, and 346 rpm. A full factorial experimental design with three replicates was run.

4.2.2.4 Rheological properties of dough

Rheological properties were evaluated with a farinograph (Brabender, Duisburg, Germany) and a Chopin NG alveograph, linked to an alveolink integrator–recorder (Chopin Technologies, Villeneuve-La-Garenne, France). Concerning farinograph assays, the standard method (AACC, 2000) was followed. Water absorption (WA, the amount of water to reach 500 BU); dough development time (DDT, time to arrive at maximum consistency); dough stability (S, time at which consistency remains stable at 500 BU); and twenty minute drop (TMD, the difference in Brabender units from the 500 line to the center of the curve measured at 20 min from the addition of water) were assessed. Water absorption was used to determine the correct amount of water for the breadmaking process (Cappelli et al., 2019a). Chopin alveograph tests followed the standard protocol ISO27971 (ISO, 2008): dough tenacity (P), dough extensibility (L), deformation energy (W), and curve configuration ratio (P/L) were evaluated.

4.2.2.5 Breadmaking process and bread quality characterization

With respect to breadmaking, the straight dough method was applied. Mixing of the ingredients, dough formation, resting, leavening with fresh brewer’s yeast, and baking were carried out with a bread machine (Pain Dorè, Moulinex, Ecully, France). Ingredients were as follows: 310g flour (type 0 following the Italian classification), 13g brewer’s yeast, 9 g salt, and a variable amount of water (ranging from 51.07% to 57.27%) determined by water absorption percentages recorded in farinograph trials (Cappelli et al., 2019). After baking, breads were cooled to room temperature and stored in paper bags, following current practice.

Regarding bread quality measurements, volume (L) was measured using the standard millet displacement method (AACC, 2000), consistent with other authors (Parenti et al., 2019). Crumb specific volume (ml/g) was measured according to the AACC method 10-05.01 (AACC, 2000). Finally, crumb and crust moisture (g/100 g) were measured by gravimetry at 105 °C until constant weight was obtained.

4.2.2.6 Statistical analysis

The effects of tempering and stone rotational speed were assessed using a fixed effects model, as described in Montgomery (2017). Flour yield, flour particle size, milling efficiency, dough rheology, and bread characteristics were tested with a two-way ANOVA with wheat moisture and stone rotational speed as the main factors, and their interaction. Significance was set at $p<0.05$. When significance was reached, a post-hoc Tukey HSD test was performed. Operative parameters optimization charts were obtained using the response surface methodology, considering wheat moisture, stone rotational speed, and their interaction.

4.2.3 Results

4.2.3.1 Milling process, energy consumption and flour particle size

Results of the milling process showed that milling time was significantly affected by stone rotational speed and wheat moisture. On the one hand, stone rotational speed decreased milling time while, on the other hand, grain moisture increased it. Consistently, mill productivity increased with stone rotational speed, and decreased with wheat moisture, ranging from a minimum of roughly 100 g/min at 17% and 173 rpm, to a maximum of about 450 g/min at 11% and 346 rpm. SE needed to obtain 1 kg of flour significantly increased with increasing wheat moisture and decreasing stone rotational speed. Thus, SE was lowest at 11% and 346 rpm, and highest at 17% and 173 rpm. This relationship between increasing moisture and SE agrees with earlier results from roller mills (Warechowska et al., 2016; Cappelli et al., 2020i) and is due to the fact that dry grain is more brittle and less resistant than humid grain (which has higher plasticity), reducing required energy.

Total yield significantly decreased as wheat moisture increased, while no significant difference was found for stone rotational speed. Maximum yield (99.6%) was found at 11% moisture content, compared to 99.0% at 17%. With regard to refined white flour yield, the individual main effects of wheat moisture and stone rotational speed were statistically significant. Yield was highest at 13% moisture, followed by 15%. Refined white flour yield decreased as stone rotational speed increased, while maximum yield was obtained at 13% moisture and 173rpm. Regarding middlings yield, the two-way ANOVA found individual main effects for moisture and stone rotational speed. Middling yield decreased as moisture increased but increased as stone rotational speed increased. Finally, with regard to bran yield, the individual main effects of wheat moisture and stone rotational speed were statistically significant. Here, bran yield increased as wheat moisture and stone rotational speed increased. Highest productivity and lowest SE were obtained at 11% moisture and 346 rpm. Thus, for both white and whole wheat flour, SE is lowest with the same settings (higher speed and lower moisture). Finally, a significant relationship was found between temperature and stone rotational speed (Table 1), where the smallest temperature increase was measured at the lowest speed (average 15.6 °C).

The water content of obtained flour fractions (i.e. white flour, bran, and middlings) increased as a function of the initial grain moisture content. Regarding particle size distribution, the obtained white flours are described by a trimodal curve, as shown in Fig. 5.

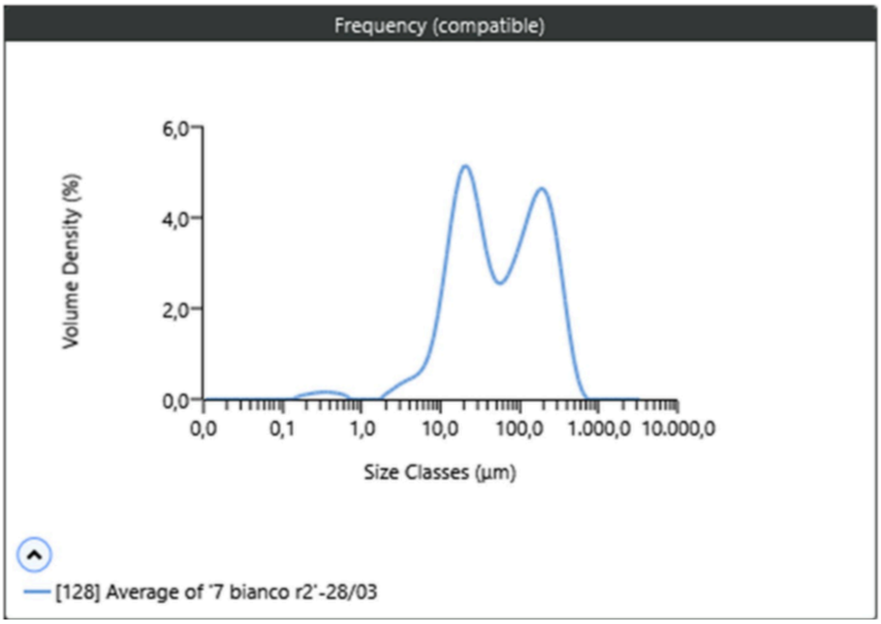

Fig 5: Graphical representation of particle size distribution in obtained flours measured with dynamic light scattering. (Source: Cappelli et al., 2020f).

A first peak was found between 0.1 μm and 1 μm in all tested flours. However, this range represents a negligible amount of total volume density. A second peak is found between 10 μm and 20 μm, and a third between 200 μm and 300 μm. Particle size distribution data are usually discussed in terms of D10, D50, and D90, namely the first decile, the median, and the ninth decile. D10 significantly changed with grain moisture – increased moisture was related to higher D10 values. Peak intensity for D50 and D90 was a function of both grain moisture and stone rotational speed, and a significant interaction was found between moisture and speed. In particular, D50 was maximum at 13% moisture and slowest speed (on average about 130 μm), and at 11% and higher speed (on average about 110 μm). D90 was maximum at 13% moisture and slowest speed (on average about 420μm). At 11% moisture, maximum D90 (about 400 μm) was found at higher stone rotational speeds. In conclusion, variation in grain moisture and stone rotational speed were able to significantly affect white flour granulometry. The following sections examine the effect of particle size distribution on both dough rheology and bread quality in depth.

4.2.3.2 Dough rheological properties

Farinograph and alveograph results are summarized in Table 5

Table 5: Results of the dough rheology assessment with the Farinograph and alveograph. Results are expressed as the mean of three replicates ± SD. Letters (a,b,c,d) represent statistically-significant differences reported by the Tukey HSD post hoc test for grain moisture, while no significant difference was found for stone rotational speed. WA = water absorption; DDT = dough development time; DS = degree of softening; TMD = twenty minute drop; P = dough tenacity; L = dough extensibility; P/ L = curve configuration ratio; W = deformation energy. (Source: Cappelli et al., 2020f).

Sample	WA (%)	DDT (min.)	DS (min.)	TMD (BU)	P	L	P/L	W
M.11% 173 rpm	57.03 ± 0.92 a	2.64 ± 0.13	3.31 ± 0.76 ab	147 ± 23	45.3 ± 2.4 a	39.7 ± 6.5 c	1.17 ± 0.12 a	55.27 ± 5.90
M. 11% 260 rpm	57.27 ± 1.33 a	2.47 ± 0.21	3.50 ± 0.43 ab	147 ± 15	43.6 ± 3.1 a	45.1 ± 4.7 c	0.98 ± 0.12 a	56.20 ± 5.39
M. 11% 346 rpm	57.03 ± 1.54 a	2.67 ± 0.14	3.42 ± 0.52 ab	150 ± 17	42.8 ± 4.3 a	38.8 ± 2.7 c	1.12 ± 0.20 a	50.47 ± 5.35
M.13% 173 rpm	56.60 ± 1.15 b	2.56 ± 0.10	3.67 ± 0.63 b	140 ± 17	43.7 ± 4.2 a	44.3 ± 5.0 bc	1.02 ± 0.21 a	56.60 ± 1.64
M. 13% 260 rpm	55.87 ± 1.25 b	2.56 ± 0.10	3.72 ± 0.50 b	150 ± 10	43.1 ± 4.0 a	49.9 ± 0.1 bc	0.87 ± 0.08 a	58.20 ± 6.90
M. 13% 346 rpm	56.27 ± 1.54 b	2.47 ± 0.29	4.08 ± 0.38 b	137 ± 21	41.6 ± 3.7 a	45.1 ± 6.4 bc	0.95 ± 0.05 a	53.60 ± 6.74
M.15% 173 rpm	53.73 ± 1.37 c	2.75 ± 0.25	3.25 ± 0.43 a	140 ± 10	36.5 ± 1.0 b	51.8 ± 1.8 b	0.72 ± 0.03 b	52.07 ± 0.64
M.15% 260 rpm	53.73 ± 1.88 c	2.64 ± 0.38	3.03 ± 0.21 a	153 ± 6	39.7 ± 3.8 b	50.5 ± 4.9 b	0.80 ± 0.04 b	56.20 ± 7.81
M. 15% 346 rpm	53.83 ± 1.44 c	2.75 ± 0.25	3.08 ± 0.14 a	150 ± 10	37.6 ± 4.8 b	51.3 ± 5.9 b	0.77 ± 0.05 b	52.27 ± 9.13
M.17% 173 rpm	51.07 ± 0.40 d	2.56 ± 0.10	2.97 ± 0.46 a	150 ± 17	40.1 ± 5.6 b	66.2 ± 3.1 a	0.62 ± 0.07 b	64.80 ± 11.34
M. 17% 260 rpm	51.50 ± 0.89 d	2.58 ± 0.14	2.83 ± 0.14 a	160 ± 10	34.7 ± 5.3 b	55.4 ± 4.9 a	0.67 ± 0.11 b	50.80 ± 7.12
M. 17% 346 rpm	51.27 ± 0.93 d	2.67 ± 0.14	2.92 ± 0.14 a	153 ± 15	36.3 ± 1.8 b	54.4 ± 4.7 a	0.69 ± 0.09 b	53.20 ± 1.91

- Farinograph results

WA decreased with increased grain moisture. WA to reach a consistency of 500 BU was maximum for 11% dough (57 g water for 100 g flour), while it was minimum for 17% dough (51 g). However, when WA and the water already contained in flours are combined, all samples needed the same amount (68 g) of water. These results are consistent with the initial moisture of flour. Dough stability was significantly affected by moisture. Between 11% and 13% it increased, reaching a mean maximum of 3.82 at 13%. Then, it decreased as moisture increased, reaching a mean minimum of 2.91 at 17%. No significant differences were found for DDT; on average samples developed in 2.6 ± 0.2 min. Furthermore, no significant differences were found for the TMD, which was, on average, 130 BU. Overall, farinograph tests established that 13% moisture was optimal since it maximized dough stability, all other conditions being equal.

- Chopin alveograph results

Analogous to farinograph results, P, L, and P/L were significantly affected by grain moisture. P significantly decreased as moisture increased: a maximum of 43.9 mm was reached at 11% moisture, compared to a minimum of 37.0 mm at 17%. Conversely, L increased linearly with increased moisture. In particular, mean L increased from 41.2 mm at 11% to 58.7 mm at 17%. Finally, the P/L ratio decreased as moisture increased. The maximum of 1.09 was reached at 11%, while the minimum of 0.66 was reached at 17%. It is important to highlight that the 0.6–0.8 P/L range is usually considered optimal for breadmaking. Here, best performance was obtained at 15% moisture. The decrease in the P/L index is an important result for weak flours, and especially Verna grain (Cappelli et al., 2018). High P/L values result in a very tenacious dough, which is difficult to work. No significant differences were found for W. This number is extremely low, and far from the values required by the breadmaking industry.

4.2.3.3 Bread quality characterization

Bread characterization is worldwide considered as one of the most important aspect in breadmaking. Bread quality results are shown in Table 6.

Table 6: Results of bread characterization expressed as the mean of three replicates ± SD. Letters (a,b,c,d) represent statistically-significant differences reported by the Tukey HSD post hoc test for moisture, while no significant difference was found for stone rotational speed. (Source: Cappelli et al., 2020f).

Sample	Bread specific volume (L/kg)	Crumb specific volume (L/kg)	Crumb moisture (%)	Crust moisture (%)
M.11% 173 rpm	2.57 ± 0.09 b	2.74 ± 0.43 b	42.52 ± 1.08 b	26.21 ± 1.32
M. 11% 260 rpm	2.72 ± 0.07 b	2.95 ± 0.47 b	42.07 ± 0.91 b	27.76 ± 1.88
M. 11% 346 rpm	2.76 ± 0.07 b	2.65 ± 0.20 b	42.95 ± 0.91 b	27.69 ± 1.18
M.13% 173 rpm	2.66 ± 0.05 ab	2.74 ± 0.34 a	42.60 ± 1.04 b	27.02 ± 1.78
M. 13% 260 rpm	2.73 ± 0.06 ab	2.46 ± 0.18 a	42.53 ± 0.41 b	27.49 ± 1.39
M. 13% 346 rpm	2.67 ± 0.08 ab	2.92 ± 0.36 a	42.15 ± 0.46 b	26.90 ± 2.81
M.15% 173 rpm	2.69 ± 0.09 a	2.86 ± 0.37 ab	41.77 ± 0.35 a	27.67 ± 1.70
M.15% 260 rpm	2.80 ± 0.05 a	2.70 ± 0.57 ab	41.67 ± 0.73 a	26.70 ± 1.78
M. 15% 346 rpm	2.76 ± 0.07 a	2.45 ± 0.26 ab	41.72 ± 0.18 a	27.84 ± 1.91
M.17% 173 rpm	2.74 ± 0.07 a	2.72 ± 0.17 b	40.79 ± 0.77 a	26.04 ± 0.21
M. 17% 260 rpm	2.80 ± 0.08 a	2.50 ± 0.37 b	40.99 ± 0.59 a	27.75 ± 1.51
M. 17% 346 rpm	2.74 ± 0.06 a	2.68 ± 0.19 b	40.31 ± 0.52 a	25.32 ± 1.57

Bread specific volume, crumb specific volume, and crumb moisture were significantly affected by moisture, while no significant differences were found for stone rotational speed. Furthermore, no significant differences were found for crust moisture. Highest specific volumes were found at 15% and 17% (2.75 L/kg and 2.76 L/kg respectively), while the minimum was found at 11% (2.68 L/kg). This is consistent with the reduction in P/L highlighted above. The decrease in moisture from 15% to 11% produced a 2.61% decrease in bread specific volume. On the other hand, maximum crumb specific volume (2.78 L/kg) was found at 11%, while the minimum of 2.63 L/kg was found at 17%. In this case, increased moisture decreased crumb specific volume of the 5.7%. Crumb moisture decreased as grain moisture increased. The maximum (42.5%) was found at 11% moisture, while the minimum (40.7%) was found at 17%.

4.2.4 Discussion

This study found that both stone rotational speed and grain moisture affected the breadmaking process in different ways. In particular, stone rotational speed affected operative parameters, while grain moisture affected almost all of the tested variables: mill operative parameters, flour particle size, dough rheology, and bread characteristics. Hence, tempering can be used to optimize bread characteristics. Bread specific volumes were highest at 15% and 17% grain moisture, while no significant decrease was measured at 13%. Bread specific volume is considered the most important parameter in baking quality: higher values are associated with better bread quality (Liu et al., 2018; Parenti et al., 2019). Dough stability was, on the other hand, maximum at 13%. Dough stability is an important indicator of dough strength (Cappelli et al., 2020f) and high stability results in a dough that is easier to knead. Furthermore, an important decrease in the P/L index (which get closer to optimal values) was highlighted for 13% and 15% grain moisture.

Wheat cultivar (Verna) resulted in a weak flour with low dough stability (lower than 5min). This time is usually considered as the minimum threshold for suitable breadmaking flour (AACC, 2000). Although tempering the wheat increased dough stability, this procedure was not able to overtook the 5 min of dough stability limit. However, tempering did maximize dough stability and bread and crumb specific volumes were maximized for 13% and 15% grain moisture. Hence, tempering did modify three characteristics of flour and the resulting bread and seems to be a powerful tool in the improvement of the breadmaking process. Finally, our results suggest that for Verna grain, optimal tempering moisture is 13–15%: this range (i) maximizes desirable characteristics, and (ii) reduces variability in the raw material. The importance of the former is easy to understand for all flours, but it becomes extremely important in weak flours, where all of the grain's potential must be exploited to improve breadmaking performance. Verna, like all wheat cultivars associated with a specific region, is highly sensitive to seasonal variation. This creates several problems in breadmaking as it becomes very difficult to develop standardized processes. Tempering can help, as it removes a source of variability (mechanical characteristics related to grain moisture), increasing the workability of these flours.

As stone rotational speed was not found to alter bread quality, the optimal speed is a function of the process. Fig. 6 shows three optima, namely: maximum yield, maximum productivity, and minimum SE for both whole and white flours.

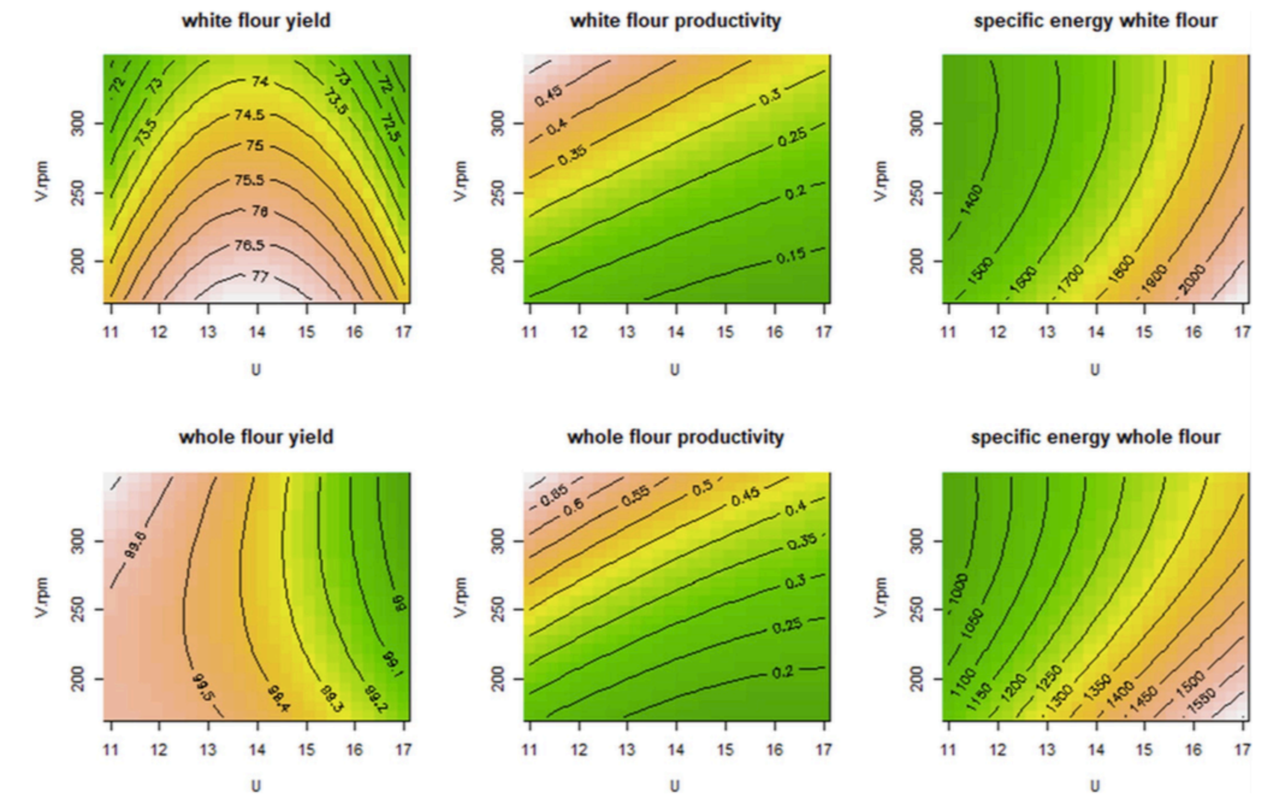

Fig 6: Optimization of the experimental mill in terms of yield, productivity and specific energy required to produce white and whole flour. Curves represent moisture (U) and speed (V) combinations for the same yield (left-hand column), productivity (middle column) and energy consumption (right-hand column) for white flour (top row) and whole wheat flour (bottom row). (Source: Cappelli et al., 2020f).

All models highlighted a high ratio of explained variance to residuals and a low ratio between lack of fit and random error. Therefore, they can be considered suitable candidates for process optimization. Maximum whole flour yield was obtained with lowest moisture at roughly 250 rpm, while increasing grain moisture to 12% required a speed higher than 300 rpm to obtain the same yield, as shown in Fig. 6. On the other hand, maximum productivity for whole flour can be obtained at lower grain moisture and higher speed, while SE is minimum at 250 rpm and 11%, or at higher speeds with a small increase in moisture. With respect to the identification of the maximum productivity for whole wheat flour, it is important to highlight that too high stone rotational speeds could generate excessive heating and losses of nutrients, therefore, it is necessary to pay attention to this parameter in mill operative setting.

White flour yields significantly increased with decreased stone rotational speeds, and changed as a function of grain moisture. Yield was lower at 11% and 17% for all speeds, and higher at 13% and 15%. Since speed affected operative parameters, but neither dough rheology nor bread quality, it can be set locally. For example, at 15% grain moisture, the best compromise between bread and crumb specific volume, and dough stability is found at higher speeds that minimize SE (maximizing productivity), while lower speeds maximize white flour yield and, at 250 rpm, whole flour yield.

4.2.5 Conclusion

This study suggests that wheat tempering and stone rotational speed are powerful tools for the improvement of breadmaking and the milling process, respectively (Cappelli et al., 2020f). Tempering affected almost all of the tested variables (mill operative parameters, flour particle size, dough rheology, and bread characteristics) (Cappelli et al., 2020f). A particularly important finding is the decrease in the P/L index for weak flours. Stone rotational speed only significantly influenced operative parameters (SE, productivity, and yield) (Cappelli et al., 2020f).

Our results suggest that the milling process is optimized by using wheat with low moisture content, milled at high speed (11% moisture and 346 rpm). Although this configuration optimizes SE, productivity, and whole wheat flour yield, it has negative effects on dough rheology and bread characteristics (Cappelli et al., 2020f). On the other hand, the results of our rheological and breadmaking tests clearly highlight that best performance is obtained by flours milled at 13% and 15% moisture. Therefore, although flours obtained from grain at 15% moisture had lower P/L values and slightly higher bread volume compared to 13% grains, the latter percentage seems to be the best compromise between milling optimization and dough and bread performance (Cappelli et al., 2020f).

At 13%, dough stability and W are higher, while bread volume and P/L are very similar to 15% (Cappelli et al., 2020f). At the same time, refined white flour yield is maximized at the lowest stone rotational speed (173 rpm) and, overall, refined white flour yield is also higher at 260 and 346 rpm, compared to the other tested moisture levels (Cappelli et al., 2020f). In conclusion, tempering can be used to improve the potential of a weak flour for two reasons: it maximizes desired characteristics, and reduces variability in the raw material (Cappelli et al., 2020f).

4.3 Improving roller milling technology using the break, sizing, and reduction systems for flours differentiation

4.3.1 Aim of the study

Grain milling might be the oldest manufacturing process in the world. This essential activity produces flour, a basic ingredient in many foods (Kweon et al., 2014; Cappelli et al., 2018). Although many techniques are used in the food industry, roller and stone mills are the most widely used (Doblado-Maldonado et al., 2012; Cappelli et al., 2020i). Selecting the optimal milling strategy is essential, as it has a significant influence on the quality of wheat flour, dough rheological properties, and bread characteristics (Doblado-Maldonado et al., 2012; Cappelli et al., 2020i; Pagani et al., 2014).

Concerning to the milling method, although stone milling is still preferred by artisanal bakers and organic food producers who appreciate its effects on flour, dough, and bread, not to mention the marketing advantage related to the use of the term “stone ground” on products, the food industry prefers roller milling (Cappelli et al., 2020i). This is because the latter has several production advantages. They include higher efficiency and flexibility (Doblado-Maldonado et al., 2012; Cappelli et al., 2020i), lower heat generation, an optimal falling number, and better dough rheology (Kihlberg et al., 2004; Cappelli et al., 2020i).

The machine–product interaction, and its effects on the final product is particularly relevant in wheat milling (Cappelli et al., 2020i; Cappelli et al., 2020f; Cappelli et al., 2020g). Although the roller mill has been significantly improved from a technological point of view, much can still be done. Earlier work highlights various interesting strategies, notably: wheat debranning (pre-milling) combined with the processing of bran and middlings (Cappelli et al., 2020i); the correct management of wheat conditioning (Cappelli et al., 2020f) and of differential ratios; and the development of fully automated, adaptive mills (Cappelli et al., 2020i). Although these solutions are appealing, another important issue is whether flour obtained from different pairs of rollers differs in terms of its nutritional content and technological properties.

The typical roller mill is divided into three systems. The first is the break system, which separates endosperm from bran and germ by opening the kernel and scraping off the bran from the endosperm (Rosentrater & Evers, 2017; Posner, 2003). Although some flour is produced, that is not the purpose of this stage (Rosentrater & Evers, 2017; Cappelli et al., 2020i). The second is the sizing system; here the purpose is to scrape bran particles from the endosperm before further processing (Rosentrater & Evers, 2017; Cappelli et al., 2020i). The third is the reduction system; here the goal is to crush and shear the endosperm until the flour meets a defined refinement standard (Rosentrater & Evers, 2017; Cappelli et al., 2020i).

There are no studies in the literature that assess or compare differences between flours obtained by the break, sizing, and reduction systems, thus motivating this work (Cappelli et al., 2020g). Therefore, the aim of this study is to assess if flours recovered from the break system, and the sizing and reduction system, differ between them and from control flours, obtained at the end of the complete milling process (Cappelli et al., 2020g). We examined two wheat cultivars: an ancient wheat (Conte Marzotto) and a modern wheat (Nogal) and assessed differences in flour yield, flour composition, dough rheological properties, and bread characteristics (Cappelli et al., 2020g).

4.3.2 Materials and methods

4.3.2.1 Raw materials, milling process and flours preparation

Investigations were carried out on two wheat cultivars, an ancient wheat (Conte Marzotto) and a modern wheat (Nogal), kindly provided by Molino Cicogni (Arezzo, Italy). The former is widely cultivated in Italy while the latter is widely cultivated in Europe. Grain was purified before conditioning, and left to stand for 24 h before milling. The milling process used a roller mill (G.L.D., Golfetto Sangati Ltd., Padova, Italy) installed at the Molino Cicogni factory in Italy, with a production capacity of 30 tons/day. As shown in Fig. 7, the break system is composed of two pairs of rollers (B1 and B2), and the sizing and reduction system consists of three pairs of rollers (C1, C2, and C3).

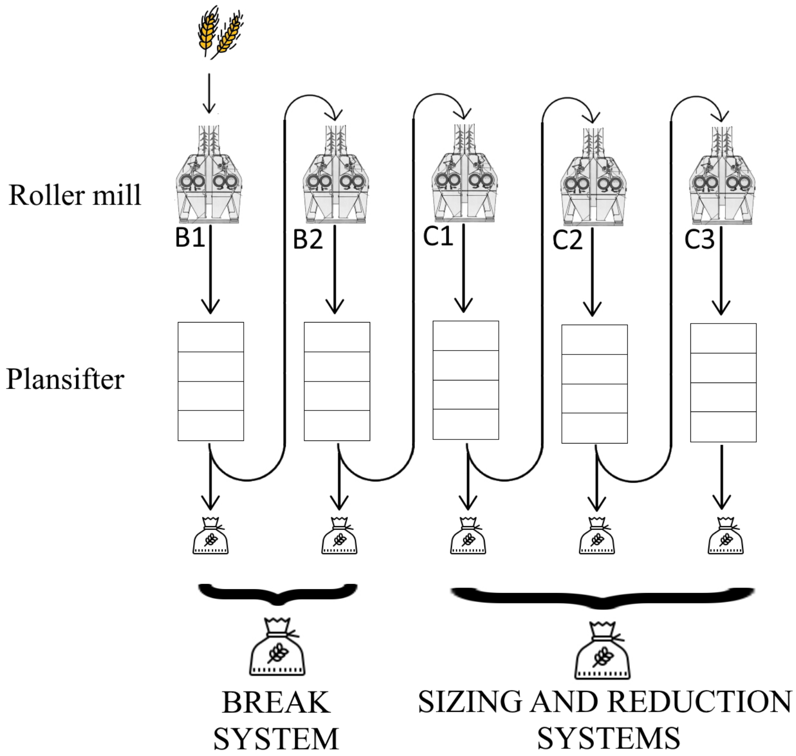

Fig 7: Schematic representation of the roller mill used in trials. (Source: Cappelli et al., 2020g).

After each passage between a pair of rollers, the milled material is sent to the respective section of the plansifter (Fig. 7). The section B1 of the plansifter is composed, in order, by six 300 μm sieves, followed by four 200 μm sieves, and finally by six 125 μm sieves. The section B2 of the plansifter is composed by six 260 μm sieves, followed by four 200 μm sieves, and finally by six 100 μm sieves. With respect to the section C1 of the plansifter, the milled material is sieved through four 160 μm sieves and six 100 μm sieves. The section C2 of the plansifter is composed by four 160 μm sieves and by six 100 μm sieves. Finally, the section C3 of the plansifter is composed by three 200 μm sieves and by six 100 μm sieves. The experiment used 100 kg of wheat for each cultivar. The refined flour obtained from each step (B1, B2, C1, C2, and C3) was collected from the respective part of the plansifter, weighed, and used to determine the total percentage yield needed to prepare the three tested flours. Three samples, each weighing 2.7 kg, were prepared for both *Conte Marzotto* and *Nogal*, as follows: the first, used the flour obtained by the break system (B1 + B2); the second, used the flour obtained by the sizing and reduction system (C1 + C2 + C3); and the third, control sample, used flour obtained by the complete milling process (B1 + B2 + C1 + C2 + C3). Fresh brewer’s yeast (Zeus, Zeus IBA Ltd., Florence, Italy), salt (Chantesel Ltd.), and water (Acqua minerale San Benedetto Ltd.) were purchased in a local supermarket.

4.3.2.2 Experimental design

Our experiment evaluated differences between flour, dough, and bread, as a function of the part of the roller mill system used to produce the refined flour. Flours obtained from the break system, the sizing and reduction system, and at the end of the complete milling process (control), were compared for both Conte Marzotto (ancient wheat) and Nogal (modern wheat). Three flours (for each cultivar) were prepared according to the percentages reported in Table 7.

Table 7: Percentages of recovered flours used in the preparation of tested flours. (Source: Cappelli et al., 2020g).

Flour sample	**B1 (%)**	**B2 (%)**	**C1 (%)**	**C2 (%)**	**C3 (%)**
Conte Marzotto control	26.84	7.45	33.25	21.57	10.89
Conte Marzotto break system	78.27	21.73	-	-	-
Conte Marzotto sizing and reduction system	-	-	50.60	32.83	16.57
Nogal control	18.38	7.94	31.90	23.50	18.28
Nogal break system	69.84	30.16	-	-	-
Nogal sizing and reduction system	-	-	43.29	31.89	24.82

Break system flour (B) was obtained by the recombination of flours recovered from B1 and B2 channels. Sizing and reduction system flour (SR) was obtained by the recombination of flours from C1, C2, and C3 channels. Finally, a control flour (C), representative of the complete milling process, was obtained by the recombination of flours from all mill channels. The percentages reported in Table 7 were determined according to milling yield. Following the procedure described in ISO 27971 (ISO, 2008), total kneading time was set at eight minutes in Chopin alveograph tests (ISO, 2008), and 20 minutes for breadmaking tests. All tests were carried out in three replicates.

4.3.2.3 Flour characterization and analysis

Protein (AOAC 920.87 (AOAC International, 2005)), total dietary fibre content (AOAC 991.43 (AOAC International, 2005)), starch (AOAC 979.10 (AOAC International, 2005)), ash (AOAC 923.03 (AOAC International, 2005)), and total phenolic content (AOAC SMPR 2015.009 (AOAC International, 2005)), were determined by the Analytical Food Laboratory (Florence, Italy) following approved, official methods.

4.3.2.4 Rheological properties of doughs

Differences in the rheological properties of doughs obtained with the three tested flours (B, SR and C) were evaluated with a Chopin NG alveograph linked to an alveolink integrator–recorder (Chopin technologies, Villeneuve-La-Garenne, France), and a farinograph (Brabender, Duisburg, Germany) in three replicates. Consistent with the procedure described in ISO 27971 (ISO, 2008), dough tenacity (P), dough extensibility (L), deformation energy (W), the index of swelling (G), and the curve configuration ratio (P/L) were evaluated. Farinograph trials followed the ICC 115/1 method from the International Association for Cereal Science and Technology (International Association for Cereal Chemistry, 1992). Here, water absorption (WA), dough development time (DDT), dough stability (S), degree of softening (DS), and the twenty minute drop (TMD) were assessed.

4.3.2.5 Breadmaking

The straight dough method was applied. Mixing, dough formation, resting, leavening with fresh brewer’s yeast and baking, were performed using a bread machine (Pain Dorè, Moulinex, Ecully, France). The recipe was as follows: 310 g of wheat flour, 13 g of brewer’s yeast, 9 g of salt, and a variable amount of water according to water absorption percentages recorded in farinograph trials. Optimum amounts of water for Conte Marzotto were: 49.60 % for C; 47.93% for B; and 49.77% for SR. In the case of Nogal, these amounts were: 51.77 % for C; 51.60% for B; and 51.47% for SR. The amount of water added in breadmaking is referred to the flour amount (310g) and not to the sum of the ingredients. Kneading was carried out at 110 RPM at 20 °C for 20 min. Resting, fermentation and proofing were performed at 40 °C for 93 min. Finally, baking was carried out at 180 °C for 48 min. After baking, breads were cooled to room temperature and stored in paper bags, following current practice.

4.3.2.6 Bread analysis

The standard millet displacement method (AACC, 2000) was used to measure bread specific volume (L/kg), consistent with earlier work (Parenti et al., 2019; Cappelli et al., 2019a). Bread loaf height (mm) was measured with a caliper at the center of the loaf. Crumb density (g/ml) was determined from the mass/ volume ratio. Crumb and crust moisture (g/100 g) were determined by gravimetry at 105 °C until a constant weight was reached.

4.3.2.7 Statistical analysis

Data were assessed with a non-parametric test. In particular, the Wilcoxon-Mann Whitney rank-sum test was applied, consistent with earlier work (Cappelli et al., 2020h). Significance was set at $p<0.05$. The statistical analysis was carried out using R software (version 3.6.1).

4.3.3 Results and discussion

4.3.3.1 The milling process

Fig. 8 and 9 report yield for the cultivars Conte Marzotto and Nogal, respectively. Furthermore, Table 7 shows the percentages of flour from each pair of rollers used in the preparation of the three tested flours (B, SR, and C).

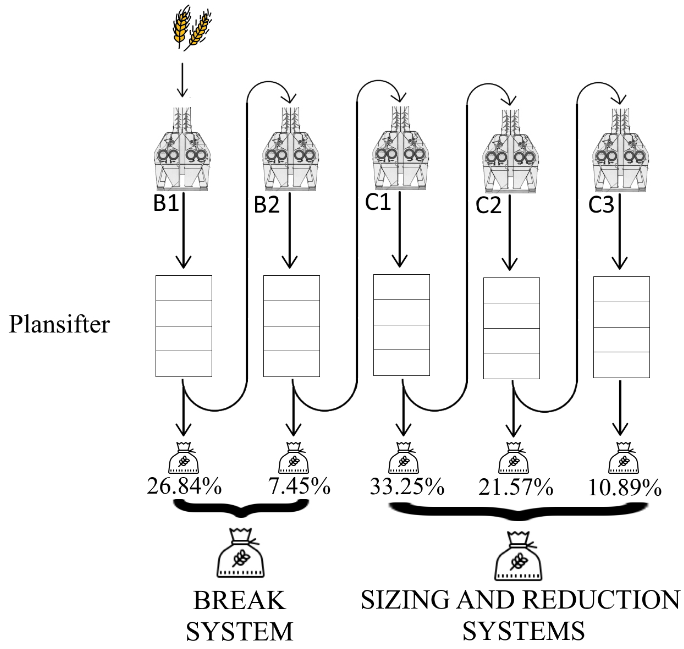

Fig 8: Yield for the Conte Marzotto cultivar. (Source: Cappelli et al., 2020g).

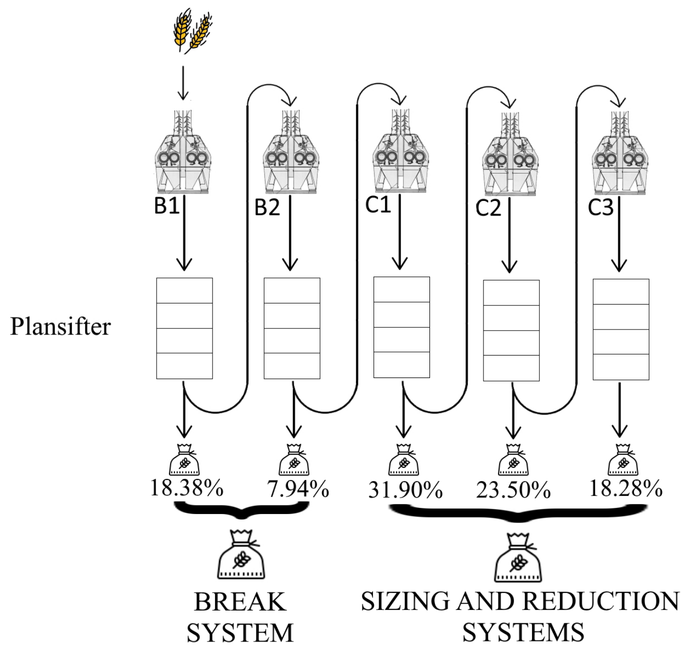

Fig 9: Yield for the Nogal cultivar. (Source: Cappelli et al., 2020g).

Fig. 8 and 9 show that yields were similar for break, and sizing and reduction systems. Regarding the break system, yield was highest in the first stage (B1) for both cultivars. Yield in the B1 channel was lower for Nogal than Conte Marzotto (18.38% versus 26.84%). This reflects the greater hardness and resistance to milling of the modern cultivar with respect to the ancient cultivar (Posner, 2003; Migliorini et al., 2016). This distinction disappeared in the second stage (B2), where yield was very similar for the two cultivars. Moreover, the differences in size or shape between wheat kernels, might be another reason which affected the flour yields.

Turning to the sizing and reduction system, yield was similar for C1 and C2 channels, for both cultivars. However, for the C3 channel, yield was higher for Nogal than Conte Marzotto (18.28% versus 10.89%). This result highlights that exposing the modern wheat cultivar to several milling stages increases refined flour yield and reduces by-products.

4.3.3.2 Compositional analyses of flour

The results of compositional analyses are shown in Table 8.

Table 8: Results of flour characterization and analyses. (Source: Cappelli et al., 2020g).

Flour sample	**Starch (g/100g)**	**Protein (g/100g)**	**Total dietary fibre (g/100g)**	**Ash (g/100g)**	**Total phenolic content (mg/kg)**
Conte Marzotto control	68.40	9.93	1.70	0.42	257
Conte Marzotto break system	69.00	9.63	1.30	0.42	237
Conte Marzotto sizing and reduction system	68.00	10.25	2.20	0.43	269
Nogal control	71.00	10.33	0.80	0.42	222
Nogal break system	73.00	10.54	0.60	0.37	220
Nogal sizing and reduction system	69.00	10.03	1.70	0.49	231

For both cultivars, B flours are characterized by lower total dietary fibre and higher starch content, compared to both C and SR flours. On the other hand, SR flours has higher total dietary fibre and phenolic content, and lower starch content compared to B and C flours. This result could be related to the aim of the milling step and the characteristics of the rollers used in break, and the sizing and reduction system, as reported in earlier work (Cappelli et al., 2020i). The slightly higher total phenolic content observed in SR flours could be attributed to the higher total dietary fibre content that is derived mainly from non-endospermic kernel components, as this is where the majority of phenolic compounds are found (Doblado-Maldonado et al., 2012). No noteworthy differences were found for other compositional parameters.

4.3.3.3 Farinograph

The results of farinograph tests are reported in Table 9.

Table 9: Results of farinograph tests expressed as mean of three replicates ± SD. (Source: Cappelli et al., 2020g).

Flour sample	Water absorption (%)	Dough development time (min.)	Dough stability (min.)	Degree of softening (B.U.)	Twenty minute drop (B.U.)
Conte Marzotto control	49.60 ± 0.10	2.67 ± 0.14	4.25 ± 0.43	110.00 ± 10.00	150.00 ± 0.00
Conte Marzotto break system	47.93 ± 0.21	2.58 ± 0.14	5.00 ± 0.25	96.67 ± 15.28	133.33 ± 28.87
Conte Marzotto sizing and reduction system	49.77 ± 0.31	2.75 ± 0.25	4.33 ± 0.52	103.33 ± 5.77	140.00 ± 10.00
Nogal control	51.77 ± 0.25	2.83 ± 0.14	5.58 ± 0.38	90.00 ± 10.00	106.67 ± 11.55
Nogal break system	51.60 ± 0.53	2.83 ± 0.14	7.00 ± 1.32	76.67 ± 11.55	93.33 ± 23.09
Nogal sizing and reduction system	51.47 ± 0.25	2.83 ± 0.14	5.67 ± 0.38	83.33 ± 5.77	93.33 ± 11.55

With respect to water absorption (WA) the results of the Wilcoxon-Mann Whitney rank-sum test do not show any statistically significant difference between the tested samples in both cultivars. Nevertheless, the figures reported in Table 9 show that, in the case of Conte Marzotto, B flour had lower WA compared to both C and SR flours. This is due to the statistically-significant relationship between WA and protein ($p < 0.001$) highlighted in earlier work (Cappelli et al., 2020h). Moreover, studies by Finney et al. (1987), Ma et al. (2007) and Kucek et al. (2017) confirm that lower protein content is related to lower WA.

Another important factor which influence WA and several others rheological parameters is the gluten quantity and quality. As widely reported in the literature, modern wheat varieties are characterized by higher protein (and gluten) content and by stronger gluten network (De Santis et al., 2017; Cappelli et al., 2020b). The latter is due to a lower gliadins/glutenins ratio (i.e. higher glutenin content), improved glutenin allelic composition (due to the introduction of high-quality alleles at the Glu-B1 and Glu- B3 loci), and the differential expression of specific storage proteins (De Santis et al., 2017; Cappelli et al., 2020b). The results reported in Table 9 support these considerations, showing lower WA in the case of Conte Marzotto B flour compared to both C and SR flours, and compared to Nogal flours.

Total dietary fibre content also influences WA; the high water retention capacity of fibre is due to the higher number of hydroxyl groups (Gómez et al., 2011). Moreover, arabinoxylans, inulin and β-glucans increase WA and have negative effects on gluten development, dough rheology, and bread characteristics (Cappelli et al., 2019a; Gómez et al., 2011; Doblado-Maldonado et al., 2012). Arabinoxylans, in particular, affect dough rheology and bread characteristics by binding water, increasing viscosity, and disturbing the formation of the protein network during kneading (Cappelli et al., 2019a; Gómez et al., 2011; Doblado-Maldonado et al., 2012). In the case of Conte Marzotto B flour, lower WA might be due to both lower protein and lower total dietary fibre content.

Regarding dough development time (DDT), the results of the Wilcoxon-Mann Whitney rank-sum test do not show any statistically-significant difference between tested samples in both cultivars. Here, in the case of Conte Marzotto, DDT values were slightly higher for SR flour compared to both C and B flours (Table 9). Like WA, this increase in DDT might be related to its higher protein and total dietary fibre content (Table 8). Studies by Miś et al. (2012) and Gómez et al. (2011) support our findings, as they highlight increased DDT with increased total dietary fibre content, due to fibre-gluten interactions that delay gluten hydration and development. Moreover, as reported in Table 9, the higher strength of the gluten network in the case of the modern cultivar Nogal, led to higher DDT values and delayed gluten development compared to Conte Marzotto (De Santis et al., 2017; Cappelli et al., 2020b).

Concerning dough stability (S), beginning with Conte Marzotto, the results of the Wilcoxon-Mann Whitney rank-sum test highlighted statistically-significant differences between B and C flours (p 0.046). Table 9 shows that

S is higher for B flour compared to the control. At the same time, no statistically-significant difference was found between B and SR flours. For the Nogal cultivar, Table 9 shows the same increasing trend in the case of B flour. Here, S was 20.29 % and 19.00% higher compared to C and SR flours, respectively. This increase in S is related to the composition of break system flours (Table 8). In particular, its higher starch (Gao et al., 2020; Sarker et al., 2008) and lower total dietary fibre content (Gómez et al., 2011), improves gluten development and dough rheological properties, resulting in an increased S. Specifically, the higher S in the case of Conte Marzotto B flour might be due to the synergistic effect of higher starch content (Gao et al., 2020) and lower dietary fibre content (Gómez et al., 2011), which guarantee an optimal gluten development.

With regard to the degree of softening (DS), the Wilcoxon-Mann Whitney rank-sum test did not find any statistically-significant differences between tested samples. Nonetheless, both for Conte Marzotto and Nogal, DS was lower for B flours compared to both C and SR flours (this is consistent with the higher values of S shown in Table 9). In the case of Conte Marzotto B flour, DS decreased by 12.12 % and 6.45 % compared to C and SR flours, respectively. These figures can be compared to decreases of 14.80 % and 8.00 % for Nogal. The same results are observed for the twenty minute drop (TMD) (Table 9). Specifically, in the case of Conte Marzotto B flour, TMD fell by 11.11 % and 4.76 % compared to C and SR flours, respectively. In the case of Nogal B flour, TMD fell by 12.50% compared to the control. The reasons for the reduction in DS and TMD for break system flours are the same as those that drive the increase in S. Higher starch (Gao et al., 2020; Sarker et al., 2008) and lower total dietary fibre content (Gómez et al., 2011) ensure correct gluten development and a dough with higher S, which, in turn, will have lower dough weakening indexes (e.g. DS and TMD).

4.3.3.4 Chopin alveograph

The results of alveograph tests are reported in Table 10.

Table 10: Results of alveograph tests (mean of five measurements (diskettes) for each proof). Results are expressed as the mean of the three replicates ± SD. (Source: Cappelli et al., 2020g).

Flour sample	P (Dough tenacity)	L (Dough extensibility)	G (Index of swelling)	W (Deformation energy)	P/L (Curve configuration ratio)
Conte Marzotto control	36.47 ± 1.21	57.80 ± 6.72	16.88 ± 1.00	69.73 ± 4.99	0.65 ± 0.10
Conte Marzotto break system	33.60 ± 1.64	64.00 ± 18.32	17.37 ± 2.58	69.80 ± 10.93	0.56 ± 0.14
Conte Marzotto sizing and reduction system	38.00 ± 2.43	54.60 ± 4.01	16.45 ± 0.62	68.80 ± 2.99	0.71 ± 0.10
Nogal control	63.53 ± 2.34	49.47 ± 2.58	15.65 ± 0.41	133.27 ± 7.16	1.23 ± 0.01
Nogal break system	65.27 ± 1.70	50.67 ± 3.06	15.65 ± 0.48	140.80 ± 7.35	1.20 ± 0.05
Nogal sizing and reduction system	62.20 ± 3.03	54.80 ± 3.42	16.87 ± 0.93	134.87 ± 8.31	1.21 ± 0.10

Regarding dough tenacity (P), the results of the Wilcoxon-Mann Whitney rank-sum test do not show any statistically-significant differences between tested samples in both cultivars. Nevertheless, in the case of Conte Marzotto, P values of B flour were 7.87% and 11.58% lower compared to C and SR flours, respectively. As reported in earlier work (Cappelli et al., 2020h), P is closely related to the protein and gluten content of flour and, here, the reduction in P is due to lower protein and gluten content of break system flours, which result in doughs with lower viscosity and tenacity (Cappelli et al., 2018; Cappelli et al., 2020f). Moreover, modern wheat varieties are characterized by higher protein (and gluten) content and by stronger gluten network (De Santis et al., 2017; Cappelli et al., 2020b). As a result, the P values of Nogal flours (B, C, and SR) are almost double of the Conte Marzotto flours (Table 10).

With respect to G and L, the results of the Wilcoxon-Mann Whitney rank-sum test do not show any statistically-significant differences between tested samples in both cultivars. Concerning G, as reported in Table 10, the results remained substantially stable. With regard to Conte Marzotto B flour, L increased by 9.69% and 14.69% compared to C and SR flours, respectively. As reported by Gómez et al. (2011), L is inversely proportional to total dietary fibre content. Moreover, according to our earlier work (Cappelli et al., 2018), it was demonstrated that an increase of starch content is linked to a simultaneous increase of L value. Table 8 shows that Conte Marzotto B flour had higher starch and lower total dietary fibre content, which explains the higher L values reported in Table 10. In the case of Nogal, L values were highest for SR flour. The latter finding might be related to differences between modern and ancient wheat characteristics and, more importantly, to a more intense milling process which produces flour with lower particle size distribution and higher L (Lapčíková et al., 2019).

Concerning W, the results of the Wilcoxon-Mann Whitney rank-sum test do not show any statistically-significant differences between tested samples in both cultivars. Nonetheless, for Nogal B flour, W increased by 5.25% and 4.21% compared to C and SR flours, respectively (Table 10). As reported in earlier work (Cappelli et al., 2020h), there is a statistically-significant relationship between W and protein content ($p < 0.001$). This correlation clearly explains the higher value of W obtained for break system flours, which had the highest protein content (Table 8). Moreover, W is strictly related to the gluten quantity and quality. As a result, W is considered as one of the most important alveograph parameters (with the P/L) by millers and bakers. As widely reported in the literature, modern wheat varieties are characterized by higher protein (and gluten) content and by stronger gluten network (De Santis et al., 2017; Cappelli et al., 2020b). in particular, the lower gliadins/glutenins ratio (i.e. higher glutenin content) and the improved glutenin allelic composition, had led to the success of modern wheat in bakery industry (De Santis et al., 2017; Cappelli et al., 2020b). The results reported in Table 10 support these considerations, showing significantly higher W values (about double) compared to the ancient wheat Conte Marzotto.

Finally, with respect to the P/L ratio, the Wilcoxon-Mann Whitney rank-sum test did not highlight any statistically-significant differences between tested samples in both cultivars. The results obtained for Nogal (Table 10) are very similar for the three tested flours. In the case of Conte Marzotto, it is important to highlight that the P/L value obtained for B flour is slightly lower compared to C and SR flours (Table 10) – in particular, it is very close to the optimal value of 0.60. This is mainly due to the decrease in tenacity (P) and to the increase of extensibility (L) in the case of doughs made with Conte Marzotto B flour, which lead to the decrease of P/L value.

4.3.3.5 Bread characteristics

Bread characterization is essential in order to assess the suitability of flours to breadmaking. Table 11 summarizes the results of the bread characterization.

Table 11: Results of bread characterization expressed as the mean of the three replicates ± SD. (Source: Cappelli et al., 2020g).

Flour sample	**Specific volume (L/Kg)**	**Crumb density (g/ml)**	**Loaf height (mm)**	**Crumb moisture (g/100g)**	**Crust moisture (g/100g)**
Conte Marzotto control	3.14 ± 0.08	0.277 ± 0.03	82.47 ± 0.38	42.40 ± 0.15	25.00 ± 0.35
Conte Marzotto break system	3.19 ± 0.10	0.273 ± 0.01	90.73 ± 3.59	42.24 ± 0.13	24.41 ± 0.16
Conte Marzotto sizing and reduction systems	3.13 ± 0.10	0.260 ± 0.03	81.00 ± 0.70	42.12 ± 0.05	24.74 ± 0.13
Nogal control	2.81 ± 0.02	0.328 ± 0.03	84.00 ± 1.82	42.77 ± 0.13	25.76 ± 0.06
Nogal break system	2.97 ± 0.14	0.323 ± 0.04	88.47 ± 5.25	42.65 ± 0.08	25.22 ± 0.10
Nogal sizing and reduction systems	2.79 ± 0.09	0.303 ± 0.04	82.63 ± 3.01	42.47 ± 0.10	25.45 ± 0.08

➢ Specific volume

The results of the Wilcoxon-Mann Whitney rank-sum test highlighted a significantly higher bread specific volume for B flour in the case of Nogal (Table 11). In particular, the specific volume obtained using B flour is significantly higher compared to the specific volume obtained using C flour (p 0.043) and SR flour (p 0.046). Although the difference was not significant for Conte Marzotto, the bread specific volume obtained using B flour was higher compared to C and SR flours (Table 11). The increase in bread specific volume in the case of break system flours is related to the lower total dietary fibre content (Table 8). As widely reported in the literature, flours characterized by lower total dietary fibre content are more suitable for breadmaking, and bread volume is higher due to lower gluten dilution and fewer gluten-fibre interactions (Fendri et al., 2016; Gómez et al., 2011). Moreover, in the case of the modern wheat Nogal, the positive effect on bread specific volume related to a lower total dietary fibre content is even more evident, since that the correct development of the stronger gluten network in modern wheats occur, better, with a lower content of disruptors (Fendri et al., 2016; Cappelli et al., 2020b; De Santis et al., 2017; Gómez et al., 2011).

- Loaf height

Regarding loaf height, the results of the Wilcoxon-Mann Whitney rank-sum test did not show any statistically-significant differences between tested samples in both cultivars. Nevertheless, the results obtained for specific volume (Table 11) were confirmed. In particular, for Conte Marzotto B flour, loaf height was 9.10% and 10.72% higher compared to C and SR flours (Table 11). Similarly, in the case of Nogal B flour, loaf height was 5.05% and 6.60% higher compared to C and SR flours (Table 11). As reported above, the higher loaf height obtained for break system flours in both the tested cultivars is due to a lower total dietary fibre content, which supports the correct development of the gluten network (with subsequent higher gas holding capacity), due to lower gluten dilution and fewer gluten-fibre interactions (Fendri et al., 2016; Gómez et al., 2011).

- Crumb density

With respect to crumb density, the results of the Wilcoxon-Mann Whitney rank-sum test did not show any statistically-significant differences between tested samples in both cultivars. In particular, values were very similar for all samples (Table 11). These results highlight that the milling system has little impact on this parameter which seems to be influenced by others factors like nitrogen fertilization of wheat, protein content of flour, and the correct management of water, bran, and middlings in breadmaking (Trappey et al., 2015; Guerrini et al., 2020).

- Crumb and crust moisture

Concerning crumb and crust moisture, the results of the Wilcoxon-Mann Whitney rank-sum test do not show any statistically-significant differences between tested samples in both cultivars. Differences were minimal, as highlighted in Table 11. As expected, it seems that crumb and crust moisture are more influenced by pre-milling operations, such as wheat conditioning (Cappelli et al., 2020f), and post-milling stages related to breadmaking, such as the amount of water and other ingredients (Parenti et al., 2020).

4.3.4 Conclusions

The results highlight that different roller mill systems significantly influence the composition of flours, dough rheological properties, and bread characteristics. The machine–product interaction can have a remarkable effect. In particular, break system flours have lower total dietary fibre content and higher starch content. On the other hand, flours recovered by the sizing and reduction system are characterized by higher total dietary fibre and phenolic content, and lower starch content.

Regarding dough rheological properties, dough stability was higher for break system flours compared to the control. Moreover, in the case of Nogal, specific volume was significantly higher for break system flour compared to C and SR flours.

These findings clearly show that each part of the roller mill system produces different flours. Millers can use this result in production, as an alternative to simply collecting flour at the end of the milling process, as in current practice. In particular, flours obtained by the break system have better rheological performance and bread characteristics. On the other hand, flours recovered from the sizing and reduction system have a more interesting nutritional profile, due to their higher total dietary fibre and phenolic content.

In conclusion, this strategy makes it possible, starting from the same batch of wheat, to use the milling process to modulate the characteristics of the obtained flours. These two flours could be sold to different markets: for example, consumers may be more interested in sizing and reduction system flours, with improved nutritional content, while bakers could be more interested in break system flours, given its better technological performance.

Finally, the application of the proposed strategy has several additional advantages; these include ease of application, no additional expenditure, no lengthening of milling time, increased profits, better product differentiation, and the expansion of the potential clientele.

5 Inter: innovations and improvements in dough rheology

5.1 Predictive models for the identification of the optimal content of bran, middlings, and water in doughs: a first application to the improvement of the rheological properties of doughs.

5.1.1 Aim of the study

Bread is considered worldwide to be an essential food for human nutrition. It is a source of energy, protein, dietary fiber, vitamins, micronutrients, and antioxidants (Boyacioglu & D'Appolonia, 1994). Recently, a significant increase in consumer interest has been observed for bakery products that are able to offer health benefits by means of bioactive compounds (Van Kleef et al., 2018). This has led to an important focus on the use of ancient grains and whole wheat flours in the food industry (Schmiele et al., 2012).

Nevertheless, wheat bran addition introduces important rheological problems in doughs (Boita et al., 2016), particularly related to the formation of the gluten network. This negative effect is related to the high concentration of fibers in bran, which is able to hold and bind a large amount of water (Li et al., 2014; Cappelli et al., 2018; Cappelli et al., 2019a), leading to a not fully hydrated gluten that has lower tenacity and extensibility (Mastromatteo et al., 2013; Cappelli et al., 2018; Cappelli et al., 2019a). More specifically, it is necessary to consider arabinoxylans, which are the principal non-starch polysaccharides present in wheat bran. These compounds compete for the usable water with gluten, starch, and the main polymers, leading to an interruption of protein aggregation behavior during heating (Rosell et al., 2010).

This behavior, which causes a migration of water from the gluten network to arabinoxylans in whole wheat doughs, has been demonstrated through nuclear magnetic resonance; a significant reduction in water absorption has been found to lead to a deficit in gluten network formation and gas-retention capacity (Li et al., 2014). In addition, a literature review reveals other problems associated with bran addition, such as restricted expansion due to destabilization of the interface between gas bubbles in fermented doughs (Cavella et al., 2008), possible piercing of gas bubbles caused by larger bran particles (Courtin and Delcour, 2002), and opposing opinions regarding the effects of bran particle size reduction on the rheological properties of doughs. The latter has been positively evaluated for an increase of bread volume (Lai et al., 1989) but has been considered negatively by many other authors (Noort et al., 2010), because of an increase in surface interaction and water absorption rate, and also as a result of the liberation of reactive compounds that diminish the aggregation of gluten-forming proteins.

Moreover, despite a more suitable nutritional profile (Dinelli et al., 2009), the poor technological properties of ancient wheats, and the addition of bran and wheat middlings highlight the need for improvements in the bread-making process, where the most important ingredient—to be added wisely—is water. In order to determine the suitability of ancient wheats, it is interesting to assess the rheological properties of doughs made with ancient wheat varieties and different degrees of refining. A second aim is to understand dough behavior given different levels of total water content and identify the optimum amount of water to be added.

Therefore, three types of flours obtained exclusively from ancient grains were investigated: refined white flour (RWF); type 2 flour (T2F), consisting of refined white flour and middlings; and whole wheat flour (WWF), containing all target fractions. T2F and WWF differ in the characteristics of their fibers and it is straight forward to evaluate potential differences related to particle size and features. The aim was to predict the rheological properties of doughs given the degree of flour refinement and total water content, starting from compositional parameters such as starch, insoluble fiber, protein, and gluten. Moreover, to optimize the production of baked products made with ancient grain flours, graphical models (level curves diagrams) were developed to identify the optimum water content of doughs in order to improve the technological properties of the finished product.

5.1.2 Materials and methods

5.1.2.1 Raw materials and flour preparation

Hard and soft ancient wheats cultivated in Tuscany were provided by Molino Paciscopi (Montespertoli, Florence, Italy). Three, five-kilo, batches were evaluated and milled to obtain the tested flours. Batches were divided into: 1) a mix of soft ancient grains (MS) composed of the following cultivars: Andriolo 15%, Frassineto 15%, Verna 15%, Gentil Rosso 15%, Inallettabile 15%, Sieve 15%, and Autonomia B 10%; 2) monocultivar Verna (MV), soft ancient grain; and 3) a mix of hard and soft ancient grains (MHS) composed of Senatore Cappelli wheat (hard ancient grain) 20%, and a mix of soft ancient grains 80%. The two blends (i.e. MS and MHS), were choosed because are the most used in the study area and they were preferred to mono-cultivars due to their more suitable technological performances; Nevertheless, mono-cultivar Verna is particularly relevant in Italy and it is often utilized in single-variety, thus motivating the MV choice.

Milling was carried out with a stone mill (model M400, produced by I.B. Manufatti ltd., Appignano, Macerata, Italy). The three target fractions (refined flour, bran, and wheat middlings) were separated by a sifter. The mean milling yield obtained in this work was 98.87%, and the mean yields of refined flour, middlings, and bran are shown in Table 12.

Table 12: Mean yields of the three target fractions (refined flour, middlings, and bran) as percentages after the milling and sieving processes of all three batches. Percentages of refined flour, middlings and bran in the three types of flour tested in this work (i.e. refined white flour (RWF), type 2 flour (T2F) and whole wheat flour (WWF)). (Source: Cappelli et al., 2018).

Type of flour	Mean yield (%)	Standard deviation
Refined flour	74.05	7.88
Middlings	14.86	6.98
Bran	11.09	0.90

Batch	Type of flour	Refined flour (%)	Middlings (%)	Bran (%)
Mix of soft ancient grains (MS)	Refined white flour (RWF)	100	0	0
Mix of soft ancient grains (MS)	Type 2 flour (T2F)	74.7	25.3	0
Mix of soft ancient grains (MS)	Whole wheat flour (WWF)	65.9	22.1	12
Monocultivar Verna (MV)	Refined white flour (RWF)	100	0	0
Monocultivar Verna (MV)	Type 2 flour (T2F)	90.9	9.1	0
Monocultivar Verna (MV)	Whole wheat flour (WWF)	81.7	8.1	10.2
Mix of hard and soft ancient grains (MHS)	Refined white flour (RWF)	100	0	0
Mix of hard and soft ancient grains (MHS)	Type 2 flour (T2F)	83.9	16.1	0
Mix of hard and soft ancient grains (MHS)	Whole wheat flour (WWF)	74.6	14.3	11.1

The three target fractions were successively recombined, according to the natural proportions found for each cultivar in the milling process, in order to obtain the three types of flour (RWF, T2F, and WWF). Table 12 shows the percentages of refined flour, middlings and bran in the three types of flour. Salt (Chantesel Ltd.) and water (Acqua minerale San Benedetto Ltd.) were purchased in a local supermarket.

5.1.2.2 Experimental design

Experimental tests aimed to identify differences among the tested doughs, as a function of five levels of total water content (70%, 76%, 82%, 88%, and 94%) and three degrees of flour refinement (RWF, T2F, and WWF). Water content was expressed as a percentage of the dry weight of the flour. A full factorial experimental design evaluated variations in rheological properties, with the three varieties of flour (MS, MV, and MHS) as replicates. Each test was performed with a Chopin alveograph, following the standard protocol ISO 27971 (ISO, 2008), which involves the preparation of five diskettes of dough for each test. The final data were the average of the five measurements.

5.1.2.3 Rheological properties of dough

Rheological properties were evaluated with a Chopin NG alveograph, assembled with an alveolink integrator–recorder (Chopin technologies, Villeneuve-La-Garenne, France). As defined in the standard protocol ISO 27971 (ISO, 2008), dough tenacity (P), dough extensibility (L), deformation energy (W), the curve configuration ratio (P/L), and the index of swelling (G) were evaluated. Chopin alveography was chosen for its capacity to simultaneously provide multiple parameters directly associated with the rheological properties of doughs, and because it is the standard method for the assessment of the technological properties of flours and doughs.

5.1.2.4 Flour characterization and analysis

Starch (ISTISAN report 1996/34 (Baldini et al., 1996)), protein (Kjeldahl method, ISTISAN report 1996/34 (Baldini et al., 1996)), insoluble fiber (ISTISAN report 1996/34 (Baldini et al., 1996)), soluble fiber (ISTISAN report 1996/34 (Baldini et al., 1996)), and gluten content (Ridascreen-Gliadin method (Pahlavan et al., 2016)) were determined by the Analytical Food Laboratory (Florence, Italy) following approved, official methods. Table 13 shows that the tested flours were characterized by relatively low protein content. This is consistent with the literature that highlights that the composition of grains and flours is strongly influenced by agronomic practices and environmental conditions (Migliorini et al., 2016). Furthermore, the addition of bran, which is characterized by a higher protein content than RWF, does not increase gluten content, despite the different protein composition (Pavlovich-Abril et al., 2016).

Table 13: Results of chemical analysis for the flours used in the trials. (Source: Cappelli et al., 2018).

Samples	Starch (%)	Protein (g/100 g)	Gluten (%)	Insoluble fiber (g/100 g)	Soluble fiber (g/100 g)	Total fiber (g/100 g)
Refined white flour, MS	85.3	11.4	5.4	3.32	2.83	6.15
Type 2 flour, MS	80.59	11.67	4.74	5.79	3.25	9.04
Whole wheat flour, MS	76.10	11.77	4.6	9.16	3.38	12.54
Refined white flour, MV	85.3	9.1	5.6	2.34	2.61	4.95
Type 2 flour, MV	79.48	9.25	5.35	5.16	2.76	7.92
Whole wheat flour, MV	73.21	9.61	5.09	8.98	3.07	12.05
Refined white flour, MHS	79.3	9.5	7.9	2.87	2.59	5.46
Type 2 flour, MHS	73.26	9.74	7.14	5.62	2.62	8.24
Whole wheat flour, MHS	69.19	9.90	6.72	8.62	2.57	11.19

5.1.2.5 Statistical analysis

Two-way ANOVA was used to test the effects of total water content and degree of flour refinement. Following Montgomery (2017) a mixed effects model employed the different cultivars as blocking factors. In this work, the relationship between alveograph parameters (G, P, L, W, and P/L) and flour components (starch, protein, gluten, and insoluble fiber) was investigated with a multiple ordinary least square regression (MOLS) (Cappelli et al., 2020h).

A predictive model of P/L and W, as a function of total water content, was developed by applying the response surface methodology to the data based on linear combinations of the variables W and P/L and their interaction (W × P/L). Goodness of fit was tested through ANOVA regression, and models were represented graphically by level curves diagrams, in which each curve represented a combination of the parameters W and P/L, associated with a determined, constant water content.

5.1.3 Results and discussion

Table 14 summarizes the results of the rheological tests and the two- way ANOVA

Table 14: Results of alveographic tests (values as means of five measurements (diskettes) for each proof with the three varieties of flour (MS, MV, and MHS) used as replications) and p-values assessed with the two-way ANOVA. (Source: Cappelli et al., 2018).

Type of flour	Water content (%)	G	P (tenacity)	L	W	P/L
Refined white	70	11.5 ± 1.7	140.8 ± 57.6	27.1 ± 8.2	149.8 ± 55.4	5.8 ± 3
Refined white	76	13.6 ± 2.7	78.3 ± 32.4	38.5 ± 15.8	101.4 ± 44.6	2.4 ± 1.4
Refined white	82	15.2 ± 1.8	55.9 ± 14.9	47.1 ± 10.9	89.4 ± 42.3	1.2 ± 0.1
Refined white	88	15.3 ± 3	27.5 ± 12.8	49 ± 18.4	45.3 ± 31.7	0.6 ± 0.3
Refined white	94	14.2 ± 2	14.6 ± 5.8	42 ± 11.8	24.5 ± 16.8	0.4 ± 0.1
Type 2	70	8.5 ± 0.6	142.7 ± 28.8	14.7 ± 2	93.7 ± 17.5	10.1 ± 2.9
Type 2	76	9.2 ± 0.9	98 ± 36.2	17.3 ± 3.3	73.8 ± 31.9	5.9 ± 2.2
Type 2	82	10.6 ± 1.6	56.3 ± 21.5	23.1 ± 6.9	53.3 ± 25.3	2.7 ± 1.2
Type 2	88	11.8 ± 2.4	34.8 ± 13.1	29 ± 10.8	39.4 ± 23.3	1.3 ± 0.6
Type 2	94	12.2 ± 3.3	19.9 ± 7.7	32 ± 15.9	24.7 ± 15.5	0.8 ± 0.4
Whole wheat	70	7.6 ± 0.7	146.3 ± 16	11.8 ± 2.1	80.2 ± 19.2	13.3 ± 1.1
Whole wheat	76	7.2 ± 0.5	109.8 ± 22.9	10.5 ± 1.3	53.3 ± 11.7	10.6 ± 2.8
Whole wheat	82	8.1 ± 0.8	85.9 ± 30.8	13.3 ± 2.6	52.7 ± 23.5	6.6 ± 2.1
Whole wheat	88	8.2 ± 0.9	57.8 ± 22.6	13.9 ± 3.1	36.2 ± 16	4.4 ± 1.7
Whole wheat	94	8.4 ± 1.1	36.1 ± 17.9	14.4 ± 3.5	24.9 ± 16.9	2.6 ± 1.1

Factor	Significance				
Water content	$p < 0.001$	$p < 0.001$	p 0.002	$p < 0.001$	$p < 0.001$
Flour type	$p < 0.001$	p 0.001	$p < 0.001$	$p < 0.001$	$p < 0.001$
Water content–flour type interaction	p 0.204	p 0.805	p 0.255	p 0.008	p 0.005

5.1.3.1 Dough tenacity (P)

With regard to dough tenacity (P), Table 14 shows that the individual effects of water content and flour type are statistically significant, while this is not the case for their interaction. P decreases with an increase in total water content. Conversely, P increases as the degree of flour refinement decreases—values for WWF are higher than for the other two flours. No significant differences are found between RWF and T2F; this highlights that the addition of middlings does not lead to significant variation in P. Variation in P, due to water content or flour type is considerably different, with water content having a far more significant impact (confirmed by the finding that variation in F-values is approximately ten times higher than for flour type).

In conclusion, and consistent with the literature (Le Bleis et al., 2015), P is found to decrease significantly with an increase in total water content. Furthermore, our study identified a significant increase in P in WWF, which indicates that the addition of bran leads to a significant increase in dough tenacity, a finding that is consistent with earlier work (Le Bleis et al., 2015). No significant differences were found for RWF and T2F. Finally, kneading time, exertion, and energy fall as total water content increases.

5.1.3.2 Dough extensibility (L)

For the extensibility index L, Table 14 shows that the effects of water content and flour type are statistically significant, while this is not observed for their interaction. Unlike P, L increases with water content and the most significant differences were observed in tests with water content between 76% and 88%. In contrast, L decreases as the degree of flour refinement decreases. Values are highest for RWF, followed, in decreasing order, by T2F and WWF. Furthermore, statistically significant differences are found between all three types of flour.

With respect to water content and flour type, variation in L is considerably different, particularly for flour type. This is confirmed by F values that are approximately twelve times bigger than those obtained for water content. The finding that L increases as total water content increases is consistent with the literature (Autio et al., 2001).

RWF has higher L values, which highlights that extensibility is closely correlated with the degree of flour refinement. More refined flours increase dough extensibility, as a function of the cultivar.

L decreases with the addition of middlings and, especially, bran. This can be explained by an increase in competition for disposable water. The addition of bran to dough creates a preferential pathway for water absorption. The presence of arabinoxylans and many hydroxyl groups steals water that would otherwise bind with gliadin and glutenin for the development of the gluten network. This competition leads to an inevitable fall in extensibility, as shown in earlier work (Rosell et al., 2010).

Despite the degradation in the development of the gluten network, the addition of bran increases total protein content, as shown in Table 13. This is because it is characterized by a higher protein content (Pavlovich-Abril et al., 2016). The different protein patterns in bran and wheat middlings means that the obtained doughs are characterized by minor gluten formation, which is weaker and leads to a significant reduction in extensibility. In conclusion, it is important to note that although these observations regarding the addition of bran to WWF are widely supported in the literature, they have not been reported for ancient grain flours; this was a motivating factor for this work.

5.1.3.3 Index of swelling (G)

The index of swelling G is related to the extensibility of doughs, like the extensibility index L. It is obtained from the square root of the volume of air, in millimeters, required to inflate a dough bubble until it ruptures. The analysis found a statistically significant difference for water content and flour type, while this did not occur for their interaction. Furthermore, Table 14 shows that G increases, albeit less than L, as water content increases. In contrast, G decreases as the degree of flour refinement decreases. Statistically significant differences are found for all three types of flour. Variation in G, as a function of water content and flour type, is considerable, notably for flour type. This is confirmed by F-values that are approximately twelve times higher than those obtained for water content. Statistically significant differences were found between RWF and flours containing other fractions of the caryopsis. This confirms a reduction in extensibility in doughs characterized by the addition of bran and middlings, due to reduced water availability.

5.1.3.4 Curve configuration ratio (P/L)

With regard to the P/L ratio, Table 14 shows that the individual effects of water content and flour type, and the water content–flour type interaction are statistically significant. It is interesting to examine this interaction in depth. The P/L ratio decreases as total water content increases. This is easily explained by opposing trends in the two parameters that make up the ratio. The decrease in P dominates the smaller increase in L, which creates a decreasing trend in the P/L ratio. The P/L index is significantly different as a function of flour composition.

The P/L ratio evaluates the equilibrium between dough tenacity and extensibility. It is crucial in the bakery industry, because it plays a key role in the technological success of leavened products. The addition of bran and middlings to doughs increases P/L values, which can only be brought back to optimal levels by the addition of a large volume of water. For RWF, optimum values of the P/L ratio were reached in tests characterized by high total water content. For T2F and WWF, it became necessary to redefine optimal intervals for P/L, which were necessarily higher with respect to those defined for RWF (between 0.4 and 0.8). The increase in the rate of variation in the P/L ratio in WWF is linked to competition for disposable water, generated by the addition of bran and middlings, which becomes less influential in tests with higher water content.

5.1.3.5 Deformation energy (W)

The dough strength index W is an alveograph parameter that is closely correlated to the technological success of bakery products, and optimum values vary according to the type of product. It is also related to optimal values of the P/L ratio. Table 14 shows that statistically significant results were found for the individual effects of water content and flour type, and for the water content–flour type interaction. W decreases as total water content increases in the tested flours, particularly RWF. The best performance, in terms of W, is obtained in tests characterized by reduced water content. The addition of bran and middlings is consistent with a remarkable reduction in W, due to a significant decrease in L, which leads to a reduction in the area under the alveograph curve. Furthermore, this reduction is linked to competition for disposable water between gluten-forming proteins and the fibrous component of bran, as found by other authors (Wang et al., 2002). This competition leads to a deficiency in the development of the gluten network in doughs made from WWF and T2F, with more significant effects seen in conditions with reduced water content. In conclusion, it is important to note that the addition of bran and middlings to doughs is consistent with a significant decrease in W.

5.1.3.6 Relationship between alveograph parameters and flour constituents

The quality and breadmaking properties of flours are closely related to their composition. In this study, each constituent (starch, soluble and insoluble fiber, protein, and gluten) was related to rheological parameters, in order to fully evaluate the interactions. Overall, the MOLS analysis identified five relationships between alveograph indexes (i.e., G, P, L, P/L, and W) and the compositional parameters of flours. The amount of variance (R2) explained by these models ranged from a minimum of 0.78 (for L), to a maximum of 0.90 (for P). Thus, the chosen chemical parameters can be considered as good predictors of alveograph parameters in these ancient wheats. Nevertheless, the rheological properties of ancient grains doughs might cover a wider range in comparison to modern commercial wheats, by virtue of which this correlation to chemical parameters might be less useful for modern grains.

These predictive models, cannot be generalized due to missing detailed knowledge of the protein, carbohydrate and fiber composition. However, such models can be considered as a starting point for the development of improved and more specific predictive models, in the near future. Furthermore, these models can be considered as powerful and useful tools for the baking industry. The results are summarized in Table 15 and discussed in the following subparagraphs, with a focus on the development of a model that is able to predict rheological behavior on the basis of compositional parameters.

Table 15: Results of the relationship between alveographic parameters and flour constituents, expressed as p-values. (−) corresponds to no statistical significance found. (Source: Cappelli et al., 2018).

Flour constituent	G	P	L	W	P/L
Starch	p 0.026	p 0.001	p 0.012	p < 0.001	p 0.041
Protein	–	p < 0.001	–	p < 0.001	p 0.036
Gluten	p < 0.001	p 0.006	p < 0.001	–	p 0.001
Insoluble fiber	p < 0.001	p < 0.001	p 0.007	–	p < 0.001
Soluble fiber	–	–	p 0.022	–	–

- Starch

Yield, protein, and gluten are routinely used as reference parameters for the determination of flour quality. In recent years, the importance of starch, which is added to several products as a functional ingredient, has become apparent. This has led to the inclusion of starch analysis in assessments of flour quality (Massaux et al., 2008). Starch is affected by many factors, such as cultivar variability and agronomic practices. The MOLS analysis found statistically significant results for the parameters G (p 0.026), P (p 0.001), L (p 0.012), W (p < 0.001), and P/L (p 0.041), referring to starch content.

It is important to highlight that starch is strongly influenced by the degree of flour refinement. As described in literature, in unrefined flours (i.e., WWF and T2F), amylase might be inactivated, which is considered as an improver for breadmaking processes (Massaux et al., 2008). In particular, amylase inactivation increases viscosity in doughs produced with unrefined flours. This could explain the increase in P and P/L and the decrease in L and G for doughs obtained with unrefined flours (Massaux et al., 2008).

Moreover, the ability of starch to influence the dough strength index W is related to its high water-holding capacity. RWF, which has high amounts of starch, is characterized by higher strength (Mastromatteo et al., 2013). This is particularly marked under experimental conditions characterized by reduced amount of total water content. In addition, it is important to highlight that in unrefined flours the dough strength decrease due to the reduction of starch content, as shown in Table 14, and for the addition of bran and middlings which lead to a modification of the protein profile.

- Soluble and insoluble fiber

The addition of bran and middlings to dough leads to significant variation in its rheological properties, notably for the different protein profile and the presence of arabinoxylans (Li et al., 2014). The results of the MOLS for insoluble fiber show a significant increase for P ($p < 0.001$) and P/L ($p < 0.001$) and a significant decrease for G ($p < 0.001$) and L (p 0.007). For W, no statistically significance difference was identified. According to Garofalo et al. (2011), an increase in arabinoxylans concentration is related to lower extensibility and higher dough viscosity and tenacity. This explains the increase in P and P/L consistent with higher insoluble fiber content in the unrefined flours tested in this work (Garofalo et al., 2011). In conclusion, the results show that insoluble fiber has a significant effect on G, P, L, and P/L, but not W. With regard to soluble fiber, a statistically significant result was found for L (p 0.022). On the other hand, no significant difference was found for G, P, W, or P/L.

- Protein and gluten

Protein content is strongly influenced by annual variability and cultivar characteristics, as reported in the literature (Migliorini et al., 2016). Another important factor that influences quality is related to the addition of bran and middlings, which are characterized by a different protein profile (Pavlovich-Abril et al., 2016). The MOLS analysis of protein is statistically significant for P ($p < 0.001$), W ($p < 0.001$), and P/L (p 0.036), but not G and L, which are related to extensibility.

As reported in the literature, high protein content is related to an increase in tenacity in doughs produced with RWF (Migliorini et al., 2016), and those obtained from unrefined flours, due to the addition of bran and middlings (Le Bleis et al., 2015); this finding explains the increase in P obtained in this work. Moreover, increases in P/L and W were found, while no significant differences were found for G and L (i.e., extensibility). This is likely to be because protein content has less influence than gluten content, particularly as earlier work (Wang et al., 2002) shows that endosperm storage proteins (gliadin and glutenin) mainly influence the extensibility of doughs.

The importance of gluten in the bread industry is widely acknowledged. In particular, the glutenin fraction is able to develop intermolecular disulfide bonds, which strongly influence the rheological properties of doughs. The MOLS analysis for gluten found statistically significant differences for G ($p < 0.001$), P (p 0.006), L ($p < 0.001$), and P/L (p 0.001), while no difference was found for W. The result for the extensibility parameters G and L is linked to the rheological contribution of gliadin, which lends extensibility to doughs. On the other hand, the significance of the tenacity index P and the P/L ratio are related to the glutenin fraction, which confers tenacity (Pena et al., 2006). Despite some reports that W is related to gluten content (Pena et al., 2006), no significant relationship was found in our dataset. However, the results reported in the literature relate to refined flours. The model suggests that unrefined flours are more affected by fiber content; therefore, the influence of fiber appears to overcome the effect of gluten.

5.1.3.7 Determination of optimal water content by graphical modeling based on the response surface methodology

Given the influence of water content on the rheological properties of doughs, three graphical models were developed to establish the optimal water content of doughs, according to the degree of flour refinement. Each curve in these models represents a combination of the parameters W and P/L associated with a determinate, constant water content. Fig. 10(a) represents the model that determines optimal water content for doughs produced with RWF. Once the type of product is defined and the values for W and P/L are set, this model identifies the optimal water content as the point where the defined values cross the nearest referenced curve.

The same applies to Fig. 10(b) and (c), which depict, respectively, the models for T2F and WWF. Goodness of fit, tested through ANOVA regression, is statistically significant for all factors, excluding the W × P/ L interaction for the model developed for WWF. With regard to T2F and WWF, given the lack of referenced values for W and P/L, further studies are needed to identify optimal intervals, in order to utilize the models in the best way.

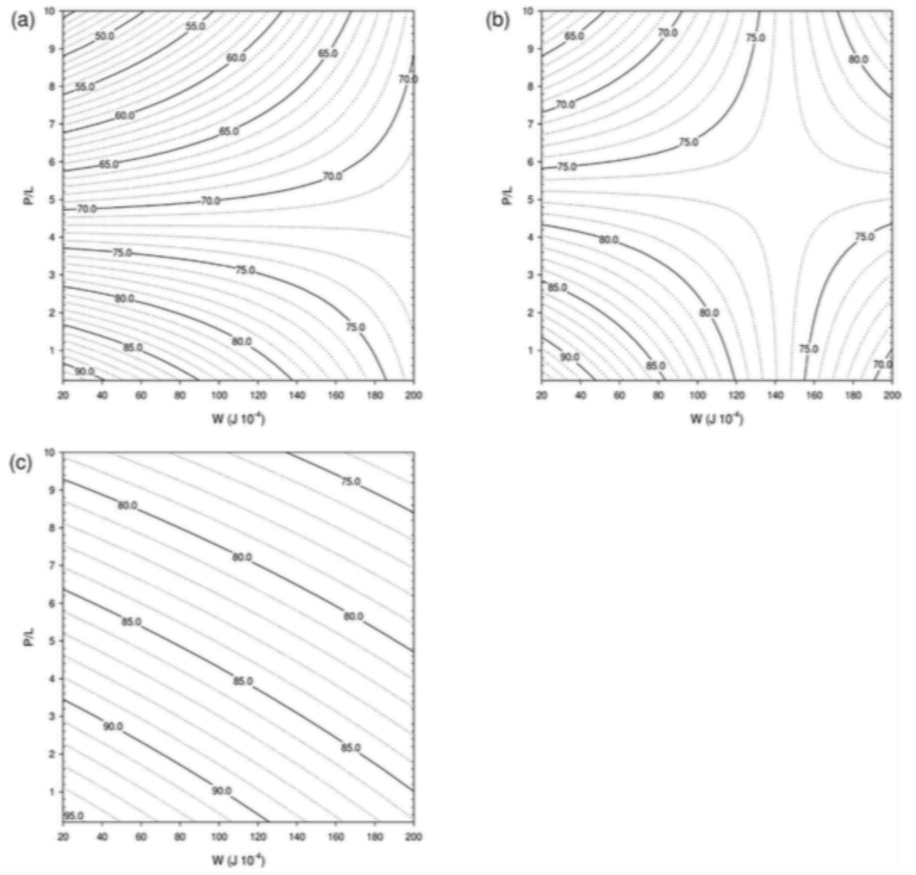

Fig 10: Model represented by a level curves diagram, which allow the optimal water content for doughs produced with refined white flours (a), type 2 flours (b) and whole wheat flours (c) to be determined. The W and P/L values change according to the type of product to be realized. The model allows the identification of the optimal water content by crossing the defined values on the axes with the nearest referenced curve, associated with a constant water content. (Source: Cappelli et al., 2018).

5.1.4 Conclusions

The results presented here demonstrate significant differences, in terms of rheological properties, between doughs made with RWF and those produced with the addition of other caryopsis fractions. An increase in the P/L ratio and tenacity, combined with a decrease in strength and extensibility in doughs made with ancient grain WWF was pointed out in our study. However, similar findings have not been reported for ancient grain flours, which was the motivation for this work. Our results confirm that data obtained from the alveographic analysis of doughs made with RWF cannot be extended to doughs characterized by the addition of bran and middlings, which is current practice.

The novelty of our work is that it makes it possible to predict rheological properties from the compositional parameters of flours, as part of a routine analysis of their suitability for bread-making, without the need for further expenditure in terms of money or time for supplementary tests. Nevertheless, this correlation might be less useful for modern grains, by virtue of which that modern commercial wheats might cover a narrower range of doughs rheological properties in comparison to ancient grains.

These refinements in the process aim to improve local production, characterized by a reduced environmental impact. They provide instruments and knowledge that facilitate the work of the agro-food chain operators and help to manage problems related to annual variability. Finally, given the increasing demand for products based on ancient grains, and despite the known technological issues, the results of our study are encouraging. In fact, the technological characteristics of the tested flours are strongly affected from both degree of refining and water content. Moreover, our research underscores the importance of establishing the optimal recipe in order to improve ancient grains baking suitability. In particular, the need to define optimum values for W and P/L in unrefined flours has not been forgotten; this will be investigated in future work.

6 Inter: innovations and improvements in dough kneading

6.1 The kneading process: a systematic review of the effects on dough rheology and bread characteristics, including improvement strategies

6.1.1 Aim of the study

The technological improvement of dough and bread is an important branch of the scientific research. Activities focus on the correct management and improvement of the unit operations making up the cereal production chain, with a “cradle to grave” approach. The following areas have been researched in greatest depth: good agronomic practices (Guerrini et al., 2020); wheat conditioning (Cappelli et al., 2020f); optimal milling methods (Cappelli et al., 2020i; Hackenberg et al., 2018a); improvements to the kneading process (Aljaafreh, 2017; Bayramov & Nabiev, 2019; Cappelli et al., 2019a; Bonilla et al., 2019; Meerts et al., 2017; Rachok, 2018; Shao et al., 2019; Sluková et al., 2017); the design of innovative kneading machines (Liu et al., 2020; Liu et al., 2017; Gao et al., 2017a; Gao et al., 2017b; Shao et al., 2019); and the assessment of dough improvers (e.g. enzymes, hydrocolloids, and emulsifiers) (Farbo et al., 2020; Ferrero, 2017; Garzón et al., 2018; Koksel & Scanlon, 2018; Tebben et al., 2018).

Starting in the field, Guerrini et al. (2020), highlighted the importance of agronomic practices, notably the correct management of nitrogen fertilization, which could improve the technological performance of flour, dough, and bread. Continuing along the production chain, the milling method has a significant impact on flour quality, dough rheology, and bread characteristics. As highlighted in an earlier review (Cappelli et al., 2020i), the most widely used milling methods are stone and roller mills. Each has its own advantages and disadvantages, and it appears to be crucial to carefully select the optimal system as a function of the aim of the process, notably business needs and demand.

Moving to the next step in the chain, a remarkable amount of research is focused on the front line – improvements to the kneading process. Here, studies have examined the use of alternative refrigerants in dough kneading, real-time measurement of kneading progress, the development of innovative kneading machines and kneading techniques, testing of potential improvers, among many other techniques. Papers have focused on the correct dosage of bran and middlings (Packkia-Doss et al., 2019), the correct management of dough water content (Meerts et al., 2017; Cappelli et al., 2018), the delayed addition of bran and middlings during whole wheat dough kneading (Cappelli et al., 2019a), the selection of enzymes, emulsifiers, and hydrocolloids (Tebben et al., 2018; Garzón et al., 2018; Farbo et al., 2020; Ferrero, 2017), and the use of alternative refrigerants, such as CO_2 snow, to control dough temperature during kneading (Cappelli et al., 2020b). The literature is, therefore, extensive, but there is a notable lack of a review that summarizes the effects of the kneading process on dough rheology and bread characteristics, and suggests improvement strategies (Cappelli et al., 2020a). The latter observation motivated the current study (Cappelli et al., 2020a)

The food industry is continuously seeking strategies and techniques that can improve the technological properties of dough and bread. Therefore, the first aim of this systematic review is to summarize current knowledge related to the kneading process, and its effects on the rheological properties of dough, and bread characteristics (Cappelli et al., 2020a). In particular, we clarify operating principles, offer a classification of kneading machines, and define key kneading parameters. The second aim is to suggest specific strategies to improve the kneading process, increasing the effectiveness, efficiency and quality of the final product, with positive impacts on production and profitability (Cappelli et al., 2020a).

6.1.2 Search strategy

The literature review explored three databases (ScienceDirect, PubMed, and the Web of Science) using the following search string:

- "Dough AND (knead* OR mix*)".

There were no language or publication status restrictions, but only results published on or after 1 January 2017 were considered. All duplicates were excluded. The initial dataset was screened by reading the title and abstract (articles that only consisted of an abstract and/ or index were excluded at this point), and then by a full text reading.

All articles concerning any aspect of dough kneading, and those that discussed it in a non-exclusive but exhaustive manner, were included, while those that did not describe machines, operating principles, or their effects on dough rheology or bread characteristics were discarded. For each database, a flow chart was produced to summarize the obtained results (Fig. 11).

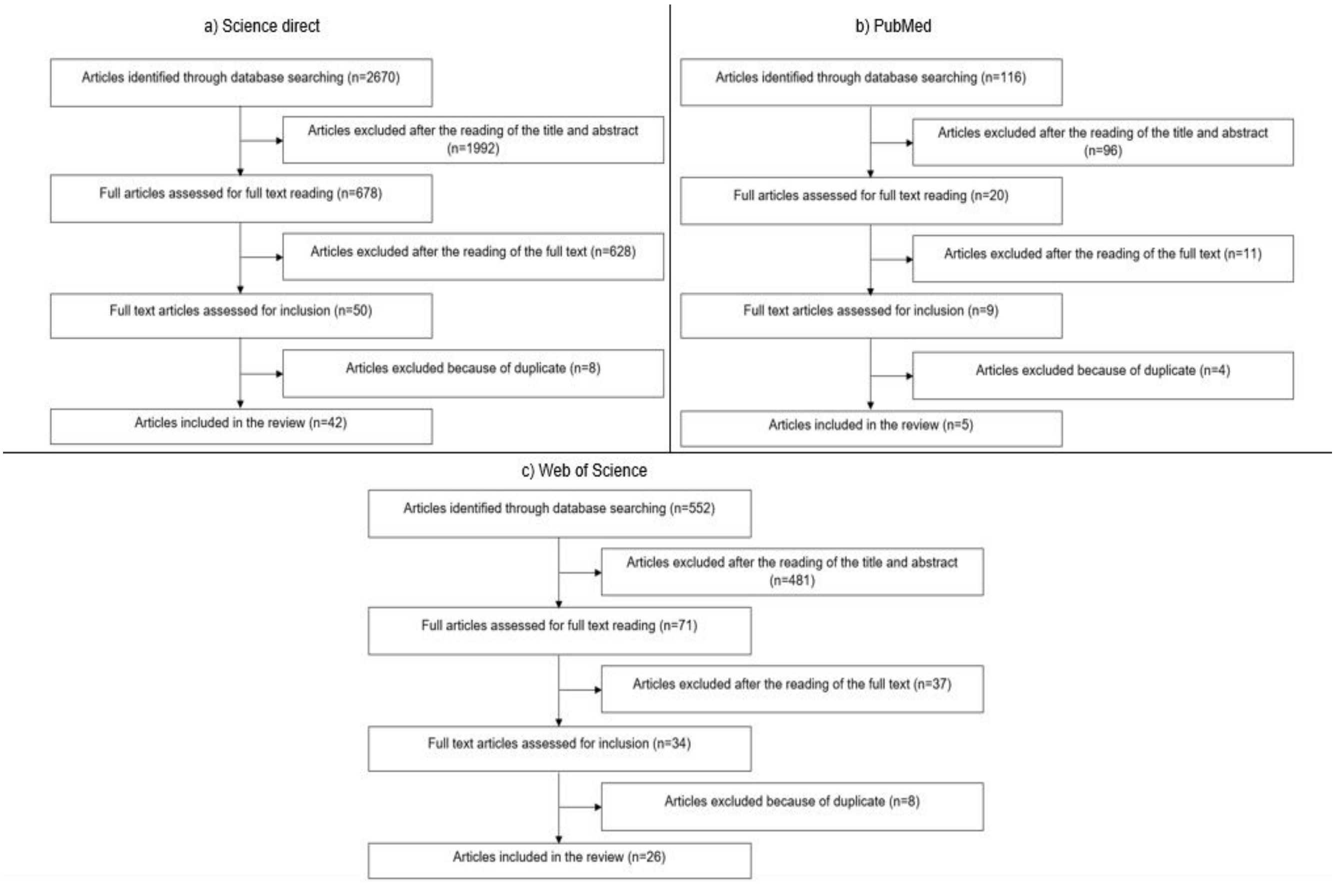

Fig 11: Flow charts pertaining to the selection process of papers on Science Direct (a), PubMed (b), and the Web of Science (c), summarizing the obtained results of the systematic literature review. (Source: Cappelli et al., 2020a).

6.1.3 *kneading process and operating principles*

6.1.3.1 The kneading process

Gao et al. (2017b) note that there are three major stages in breadmaking: kneading, fermentation (i.e. proofing) and heating (i.e. baking/ steaming). The mechanical energy imparted during kneading induces the formation of a viscoelastic dough matrix. In addition to the proper development of the gluten network, the initial inclusion of gas during mixing, and yeast activity during proofing, also greatly affect bread quality. The combined effects of gas production and retention largely determine loaf volume, crumb structure, and the texture of the bread. At the same time, the development of the gluten network is key, as gas retention capacity is essentially due to the presence of a strong and elastic network. Bread volume is a key determinant of bread quality, with higher volumes usually being preferred.

Kneading (or mixing) is a fundamental unit operation in most food industries. It increases homogeneity by reducing nonuniformity or gradients in mixtures of two or more components (Berk, 2018; Fellows, 2016). Mixing of liquids, solids and/ or gases has a wide variety of applications, for example: to combine ingredients; prepare syrups and brines; and dissolve or disperse materials (Berk, 2018; Fellows, 2016). The sole aim is to modify functional properties (Fellows, 2016). The main effect is to increase the uniformity of products by evenly distributing ingredients throughout the bulk (Berk, 2018; Fellows, 2016). Although it has no direct effect on the shelf-life of a food, it may have an indirect effect by causing mixed components to react, and the effect may be accelerated if significant heat is generated in the kneading machine (Fellows, 2016; Gally et al., 2017).

Kneading is followed by forming, a sizing operation in which foods that have high viscosity or a dough-like texture are molded into a variety of shapes and sizes before proofing and baking (Gally et al., 2017; Cauvain, 2019; Gibson, 2018; Ishwarya et al., 2017; Fellows, 2016; Kokawa et al., 2017). In some breadmaking processes, forming might be preceded by resting and/ or further leavening (Niccolai et al., 2019).

6.1.3.2 Operating principles

The main functional components of wheat flour are gluten, starch, and pentosans (a.k.a. arabinoxylans) (Kweon et al., 2014). These three components, with the addition of water and mechanical energy, can form complex network systems in doughs (Kweon et al., 2014). Starch granules are dispersed within a continuous network made up of gluten proteins (Brandner et al., 2019; Sangpring et al., 2017). Although starch dominates, the gluten structure is mainly responsible for the overall, viscoelastic properties of dough (Brandner et al., 2019). Gluten is composed of gliadins and glutenins (Ooms & Delcour, 2019; Wang et al., 2020). Viscosity and extensibility are determined mainly by gliadins, whereas toughness and elasticity are determined by glutenins (Brandner et al., 2019; Ooms & Delcour, 2019; Wang et al., 2020). Glutenins are classified into high molecular weight (HMW) and low molecular weight (LMW), based on their molecular mass and functionality (Bonilla et al., 2019; Wang et al., 2020). HMW glutenins have been shown to have a higher impact on dough elasticity than LMW glutenins, while the latter have been shown to contribute to dough strength (Bonilla et al., 2019; Wang et al., 2020). Polymers are stabilized by a combination of forces, notably intra- or intermolecular disulfide and/ or hydrogen bonds, and ionic or hydrophobic interactions (Brandner et al., 2019; Quayson et al., 2016; Ooms & Delcour, 2019; Hackenberg et al., 2018a).

The gluten network is formed when wheat flour and water are mixed, and mechanical energy is applied (Tietze et al., 2019; Preedy & Watson, 2019). Initially, the components are distributed, then hydration of flour particles is initiated, and protein aggregates in the flour become increasingly sheared and stretched. This viscoelastic network determines most dough properties on a molecular and microscopic level (Jekle et al., 2019). Mechanical energy comprises tension, compression, and/ or shear, and is transferred from the kneading element to the dough (Tietze et al., 2019). During mixing, dough is exposed to large uniaxial and biaxial deformation, and a continuous protein network is formed (Preedy & Watson, 2019). Kneaders with spiral hooks incorporate mechanical energy mostly by tension and compression, while shear dominates in the rotating blades of high-speed mixers (Tietze et al., 2019). In many mixing processes, including dough, as mixing energy increases, resistance to extension increases and then, after a certain point, decreases (Aljaafreh, 2017). Jekle et al. (2019) note that once an optimum point is passed and mechanical input to the system exceeds a certain limit, the gluten network becomes overstressed, weakening the whole system. Aljaafreh (2017) observe that high-quality bread is obtained by stopping mixing close to maximum resistance. In practice, a change in resistance causes a change in motor torque, which affects power requirements. Therefore, the rheological characteristics of the mixed material are related to the torque of the mixing machine (Aljaafreh, 2017; Asaithambi et al., 2020).

Dough formation is the result of physicochemical processes that develop simultaneously. These processes are a function of the duration and intensity of mixing, the trajectory of the movement, the configuration of the working element of the mixer, dough temperature, and the quality and quantity of ingredients (Bayramov & Nabiev, 2019). For most bakers, mixing is the most decisive step in determining final bread characteristics, as it is the step they have most control over (Asaithambi et al., 2020; Gao et al., 2017a; Gao et al., 2017b). The correct management of the kneading phase is essential for four reasons. First, it is the key to obtaining high-quality dough and bread (Kokawa et al., 2017); managing mechanical energy can lead to the breakage and/ or formation of both covalent and non-covalent bonds (Sluková et al., 2017; Gao et al., 2017a; Gao et al., 2017b). Second, it makes it possible to dose the amount of air bubbles in the dough (Sadot et al., 2017; Gao et al., 2017a; Gao et al., 2017b; Asaithambi et al., 2020). Third, it is possible to improve both dough and bread by dosing the ingredients (water included), and deciding when to add them (Cappelli et al., 2019a). Fourth, modern kneading machines make it possible to manage dough temperature, which is crucial for dough development and bread performance (Quayson et al., 2016; Cappelli et al., 2020b).

6.1.4 Types of kneading machines

There are five main types of kneading machines, each suited to particular products and applications: vertical spindle mixers, high-speed horizontal mixers, double arm mixers, continuous mixers, and planetary mixers (Davidson, 2018; Berk, 2018). Each of these types are described in the following sections.

6.1.4.1 Vertical spindle mixers

Davidson (2018) outlines the process. Vertical spindle mixers use a mobile dough tub into which ingredients are fed manually or automatically from hoppers. The dough tub is then taken to the mixer and either the vertical spindles are lowered into the tub, or the tub is lifted to the spindles. The dough is mixed in the dough tub and, after each mix, the tub is transferred to the fermentation room. After fermentation, the dough tub is brought back to the mixer for the final stage of the mixing cycle. In such a two-stage mixing process, additional ingredients are added before the final mix. The mixing action is slow and thorough and generates little heat. This type of kneading machine is primarily used to produce crackers, mainly of the sponge-and-dough, fermented kind.

6.1.4.2 High-speed horizontal mixers

The following details are taken from Davidson (2018). Dry ingredients may be fed from an automatic feeding system mounted above a stainless steel mixer bowl, while water and other liquid ingredients are metered and fed automatically. Typically, small ingredients are fed by hand, but they can also be fed automatically from a small hopper located above the bowl. The bowl is equipped with a water jacket, which cold water circulates through, and it can tilt to discharge the mixed dough. The tilt may fully invert the bowl and automatically discharge the dough directly into a hopper located below the mixing floor that feeds the sheeter of the forming equipment.

The mixer blade provides a vigorous extruding and shearing action to develop the gluten, which results in an increase in temperature. Dough temperature is monitored by a thermocouple in the wall of the mixer bowl. The shaftless mixer blade design gives an end-to-end mixing action that incorporates all ingredients evenly, including those added in small quantities. The method avoids dough adhering to a central shaft, which would result in it not being fully incorporated into the dough mass. This type of kneading machine is mainly used in cookie snack production.

6.1.4.3 Double arm mixers

Very popular in Italy, and also known as ARTOFEX, double arm mixers appeared in the early 1900s. The machine is characterized by two arms whose movement (both in a circular and vertical sense) simulate the movement of human arms. The design is suited to very hydrated doughs that require good final oxygenation to fully develop the gluten network. The movement of the arms, from top to bottom, increases dough oxygenation, thus increasing dough volume compared to planetary mixers. Although best-suited to the preparation of bread dough, this kneading machine is perfect for the production of several (highly-leavened) Italian Christmas and Easter sweet bakery products (such as panettone and colomba). It has several, key advantages: more oxygenated and voluminous doughs, minimum heat transfer, shorter average kneading time, and the ability to adjust the height of the arms to make it easier to separate the dough from the bowl and the arms. Its bulk, high cost and lower productivity are the main disadvantages.

6.1.4.4 Continuous mixers

Davidson (2018) gives details of continuous mixers. These machines are successfully used for a wide range of doughs. They are supplied with fully automatic ingredient feed systems and are usually found on single-purpose production lines. The continuous mixer combines all minor ingredients, and a portion of the required flour in the first mixing stage. In this stage, special mixing elements create a homogeneous dough. In the final stage, the remaining flour is added. This kneading machine is ideal for the production of bread, crackers, biscuits and cookies.

6.1.4.5 Planetary mixers

The following is taken from Fellows (2016). These machines take their name from the path followed by the rotating blades that include all parts of the vessel in the mixing action. Blades may be located centrally or offset from the center. In both types, there is a small clearance between the blades and the vessel wall. Blades are used for mixing pastes, blending ingredients and the preparation of spreads; hooks are used for dough mixing; and whisks are used for batter or sauce preparation. Another design combines a planetary blade and a high-speed dispersion blade. Both agitators have independently variable speeds and rotate on their own axes around the vessel. The planetary blade feeds material into the high-shear zone of the orbiting high-speed disperser. Double planetary mixers have two vertical mixing blades, and mixing is reported to be 30% faster than with other planetary mixers.

Davidson (2018) gives further details. The mixing bowls for planetary mixers are mobile, and ingredients are fed remotely. The system uses one or two mixing tools with a scraper, which are rotated on a preset configuration adapted to the product. Mixing speed and rotation can be set separately. Planetary mixers may have a facility to inject air into the mix to create a light, low-density dough. This kneading machine is very versatile and is used for the production of bread, crackers, biscuits, cookies and other baked products.

6.1.5 Results of the systematic literature review

The initial dataset consisted of 3338 texts. This was reduced to 93, following the application of the selection criteria reported in the section 6.1.2 (search strategy). The removal of duplicates left a final total of 73 items: five book chapters, eight reviews, and 60 research papers. Fig. 11 summarizes, in the form of flow charts, the selection process, which is consistent with the PRISMA statement (Moher et al., 2009). Fig. 1(a), (b), and (c) show results for ScienceDirect, PubMed, and the Web of Science, respectively.

6.1.6 Key parameters, and their effects on dough rheology and bread quality

The review of the literature concerning dough kneading reveals that the correct management of some key parameters allows to significantly improve the process and its effects on dough rheology and bread characteristics. Therefore, kneading time, dough temperature, mixing speed, dough aeration, water absorption of flour, water temperature, and total water content of dough all need to be carefully managed as a function of the product to be produced. In the following subsections, the key parameters in dough kneading are described.

6.1.6.1 Kneading time

Kneading time is an important parameter in gluten network formation and dough aeration (Gao et al., 2017a; Sluková et al., 2017; Kokawa et al., 2017; Liu et al., 2020; Meerts et al., 2017). In particular, it has been found to have a significant impact on gas release kinetics and the development of the gluten structure (Gao et al., 2017b; Sluková et al., 2017; Kokawa et al., 2017; Liu et al., 2020). Under optimal mixing and fermentation conditions, dough develops desirable viscoelastic properties and expands in the oven (oven spring), resulting in a bread with a desirable volume, gas cell structure, and flavor. In general, longer mixing and fermentation times result in softer breads (Kokawa et al., 2017; Liu et al., 2020) and longer fermentation times are preferred for breads that are mixed for a short time. However, many other properties are affected (Kokawa et al., 2017; Hackenberg et al., 2018b).

Bonilla et al. (2019) observe that, during mixing, the three gluten subunits (LMW glutenins, HMW glutenins, and gliadins) associate to form a strong gluten network, and they state that understanding the distribution of these subunits during mixing is useful in understanding the dough development mechanism. Their farinograph-based analysis of arrival time, peak time, and departure time highlighted that at arrival time, gliadins made up 17.55% of the dough, compared to 3.43% and 4.40% for LMW and HMW glutenins, respectively (Bonilla et al., 2019). The authors argued that this was due to the higher mobility of smaller-molecule gliadins, and the absence of intermolecular disulfide bonds (Bonilla et al., 2019). As mixing time was increased to match peak time, LMW and HMW glutenins indicated fewer protein agglomerates and a more network-like structure (Bonilla et al., 2019). These changes in network analysis parameters indicated network development throughout dough mixing, from arrival time to peak time. Gliadins distribution was found to be more even at peak time than other mixing times. Their quantitative network analysis of LMW and HMW glutenins found a significant increase compared to gliadins from arrival to peak time (Bonilla et al., 2019). LMW glutenins were able to spread further than HMW glutenins. This might be due to differences in molecular mass and functionality, since the former are considerably smaller and more mobile than the latter (~30–50 kDa vs. ~70–100 kDa), and the latter have a much higher density of intermolecular disulfide bonds.

The same study from Bonilla et al. (2019), found significant amounts of glutenin macropolymer in under-mixed doughs, but none in optimally-mixed doughs. The findings showed that at peak time (higher dough strength), the co-localization of HMW glutenin with gliadins (0.84) was significantly higher than the co-localization of LMW glutenin with gliadins (0.62) (Bonilla et al., 2019). While both glutenins significantly increased from arrival to peak time, they were not significantly different at arrival time. Bonilla et al. (2019) argued that the statistically-significant higher co-localization between HMW glutenins and gliadins, compared to LMW glutenins and gliadins at peak time indicated that HMW glutenins have more impact on the distribution in HMW glutenin subunits, leading to a higher dough strength at peak time. In general, they found an expected fall in quantitative network parameters as dough strength fell from 510 BU to 480 BU (Bonilla et al., 2019). Decreases in other parameters (e.g. network junctions and end points) were also of larger magnitude for LMW than HMW glutenins, and gliadins from peak time to departure time. For instance, the average number of network junctions fell from 468 to 99 for LMW glutenins, from 258 to 130 for HMW glutenins, and from 675 to 333 for gliadins (Bonilla et al., 2019).

Furthermore, HMW glutenin appears to play a major role in the gluten network and dough strength. LMW glutenins have fewer intermolecular and intramolecular disulfide bonds; these are broken down more easily by mechanical energy during mixing and aggregate in a few areas (Sluková et al., 2017; Bonilla et al., 2019). During the early stages of mixing (arrival time) LMW and HMW glutenins form aggregates. The latter are distorted and form a uniform network with gliadins when mixed until the dough reaches peak time (Sluková et al., 2017; Bonilla et al., 2019). With further mixing, the network breakdown and consequent decrease in dough strength occurs in two phases (Sluková et al., 2017; Bonilla et al., 2019). At first (peak time to departure time) LMW glutenins dissociate from the network and form aggregates. The second phase follows departure time, and is marked by an ongoing decrease in dough strength. HMW glutenin dissociates from gliadins and forms aggregate that is co-located with LMW glutenin aggregate. It appears that these changes in the aggregation of HMW glutenins is the key factor in the continued network disruption and decreased dough strength.

6.1.6.2 Dough temperature and mixing speed

Part of the mechanical energy resulting from the mixer agitator rotation dissipates into heat. Heat is transferred to the dough and the atmosphere (Sadot et al., 2017; Bayramov & Nabiev, 2019). Sadot et al. (2017) observe that part of this energy helps to develop the gluten network and gives dough its rheological properties, notably its gas retention capacity. As the gluten network forms, dough becomes increasingly resistant and the power needed to maintain the rotation speed increases, until the gluten network reaches maximal cohesion. However, when dough is overmixed, it tends to become sticky and lose its viscoelastic properties. The authors tested the effect of an increase in rotation speed, and found that it increased the speed of gluten network formation. They reported that at a higher rotation speed, fewer spiral agitator revolutions were needed to reach maximal power during kneading.

Sangpring et al. (2017) also found that mixing speed affected dough development; specifically, increasing speed increased dough consistency, irrespective of the mixing temperature. Moreover, mixing temperature was observed to have a greater impact on dough consistency and stability than speed, and different energy inputs changed dough consistency (Sangpring et al., 2017; Quayson et al., 2016). Bayramov and Nabiev (2019) observed that mixing was optimal when a preset dough temperature was reached. When dough mixing is intense, the machine's blade cuts through the dough or pushes it towards the walls of the chamber, increasing the temperature (mainly due to friction); higher temperatures can lead to irreversible protein denaturation. In high-speed dough mixers, the process is accompanied by a rise in temperature of 5–7 °C and, in superhigh-speed mixers, this increases to 10–20 °C. Given that protein swelling peaks at 20–30 °C, a further temperature increase decreases gluten swelling. The fall in gluten swelling at temperatures above 30 °C is explained by its denaturation. Swelling is not only osmotic, it is also due to solvation of the hydrophilic groups of protein micelles. Although weak gluten swells quickly, it ultimately swells less. In contrast, strong gluten swells more slowly, but eventually swells more.

Mixing conditions are very important; it is advisable to knead at a low temperature (i.e., 17 °C) and prolong fermentation to improve baking performance (Preedy & Watson, 2019; Quayson et al., 2016; Cappelli et al., 2020b). Dough should be mixed for an optimum time to develop fully (Preedy & Watson, 2019); under-mixing may leave small, unmixed patches that interfere with the proofing stage, whereas excessive mixing leads to slack and sticky dough, due to weakening of the protein network (Preedy & Watson, 2019). A low dough temperature has been found to improve gluten development (Cappelli et al., 2020b; Quayson et al., 2016). In particular, low temperatures strengthen and increase hydrogen bonds which, although weaker than disulfide bonds, are present in higher numbers and play a key role in the stabilization and strengthening of the structure (Cappelli et al., 2020b; Quayson et al., 2016). At the same time, low temperatures promote the formation of macromolecular aggregates, improving gluten development (Cappelli et al., 2020b; Quayson et al., 2016).

6.1.6.3 Water temperature, water absorption, and water content

Kweon et al. (2014) and other authors pointed out that the water temperature is an essential parameter which significantly influences gluten development, dough rheological properties, and the final quality of bakery products. In particular, Kweon et al. (2014) emphasized that the production processes of certain bakery products require hot water in dough-mixing, in order to promote and enhance the gluten development. This is due to the technological properties of hot water, which is a far more effective and efficient plasticizer of gluten than is colder water (Kweon et al., 2014). For this reason, the higher is the water temperature, the greater will be the plasticization effect of hot water (Kweon et al., 2014). Moreover, hot water influences sugars dissolution. In particular, the hot water used in dough-making allows sugars to dissolve completely during kneading (Kweon et al., 2014). Furthermore, dough properties are affected by the interactions of flour, water, and air. The interaction of flour and water makes water the second most important ingredient after flour (Kweon et al., 2014; Yang et al., 2019). The capacity of flour to retain water is, therefore, an important parameter that influences the rheological properties of dough and the quality of bread. Flours that can absorb more than 60% of their weight in water produce a slow-fermenting bread dough that maintains its shape during the final fermentation and baking. Flours that absorb less than 54% of their weight in water produce dough that forms quickly, but rapidly degrades during final fermentation, and produces a poorer-quality finished bread product. Flour particle size has been found to influence its water absorption capacity, the rheological characteristics of gluten and dough, and amylolytic enzyme activity. It is well-known, both from theory and, especially, everyday practice, that adding too much water to flour generates a soft and sticky dough. At the same time, dough with a moisture content that is below the optimal absorbency of the flour will be harder to knead and require more work in the production line. This observation is supported by the results of earlier work (Cappelli et al., 2018) which highlighted that an increase in total water content of dough increases dough extensibility (alveograph L) and the index of swelling (alveograph G), and decreases dough tenacity (alveograph P), deformation energy (alveograph W), and the curve configuration ratio (alveograph P/L). Conversely, a poorly hydrated dough would consistently show increases in P, W, and P/L, and a decreases in G and L.

6.1.6.4 Dough aeration

Dough aeration is a very important parameter in the breadmaking process because it contributes to oxidation and initiates proofing by entrapping air bubbles. Sadot et al. (2017) observed that the rotation speed of a spiral agitator affected air incorporation; the higher the rotation speed, the greater the amount of air in the dough. Each revolution incorporates air as the spiral agitator brings different parts of the dough together. A higher rotation speed means more revolutions in the same time and, therefore, more incorporated air. The latter authors found that the quantity of air incorporated in dough was directly correlated to the number of revolutions. At the same time, gluten proteins have been found to be much less susceptible to overmixing in an oxygen-lean environment, which demonstrates the significant role of oxygen in degradation (Meerts et al., 2017). The latter authors highlighted that, if allowed time to rest, overmixed dough can shows signs of recovery.

6.1.7 *Strategies to improve kneading*

Section 6.1.6 outlined several key parameters that influence kneading. The literature emphasizes that correct management of the process is able to significantly influence dough rheological properties and bread characteristics. Consequently, the following subparagraphs outline some strategies to improve the kneading process (section 6.1.7) and kneading machines (section 6.1.8).

The most interesting strategies to improve kneading are:

6.1.7.1 Maintain a low dough temperature

Asaithambi et al. (2020) note that dough temperature increases with mixing time, mainly due to the heat generated by the frictional force. Although some of this can be attributed to the dissolution of flour in water, the contribution remains negligible. They report that the maximum acceptable final temperature is 27 °C, above which the dough becomes sticky. This finding is supported by Quayson et al. (2016). The latter authors tested the effect of temperature on hard and soft wheat doughs mixed at 4, 15, and 30 °C. The study found that protein features of hard wheat flour did not change as mixing temperature decreased, the only exception being an increase in SDS-accessible thiols. However, lowering the mixing temperature for soft wheat flour led to an increase in SDS protein solubility and SDS-accessible thiols, together with an increase in b-turn structures at the expense of b-sheet structures. Thus, noncovalent interactions appear to drive protein network formation at low temperatures (4 and 15 °C), while covalent interactions dominate at standard mixing temperature (30 °C) in doughs made from both flours.

AACC International standard temperatures for straight dough breadmaking (AACCI Approved Method 10-10.03) and the farinograph test (AACCI Approved Method 54-21.02) (AACC, 2000) are 29 and 30 °C, respectively. Doughs mixed at temperatures below 30 °C must be mixed for longer to achieve the same development as conventional dough mixed at 30 °C (Quayson et al., 2016). Moreover, low-temperature production is more expensive as techniques such as water jackets and the maintaining a cooler ambient temperature must be deployed (Quayson et al., 2016). On the other hand, dough is less sticky, and the resulting loaf has higher specific volume and better texture compared to conventional mixing conditions (Quayson et al., 2016; Cappelli et al., 2020b). The results published by Quayson et al. (2016) indicate that mixing temperature has a significant effect on protein structure characteristics and dough quality. A low dough temperature during kneading appears to be essential to guarantee correct development and bread quality. These findings are supported by Cappelli et al. (2020b), who assessed the effect of CO_2 snow addition on dough thermoregulation and bread characteristics. The latter study found that the addition of high percentages of CO_2 snow (up to 10%) maintained a low dough temperature during kneading, and increased bread specific volume and loaf height.

6.1.7.2 Measuring kneading progress in real time

Real-time measurement of the progress of kneading is essential for efficient process control, in order to optimize dough rheological properties and bread characteristics. Aljaafreh (2017) reports that the rheological characteristics of the mixed material usually reflect the density and viscosity of the mix. Many variables can affect the end time of the process, dough rheological properties, and bread quality; these include the quantity and temperature of the mixture, and the temperature of the environment. The latter author notes that many of these variables are difficult to model or control.

Sangpring et al. (2017) propose a strategy based on colorimetric indexes. The authors measured changes in systems using a color difference meter. They tested that addition of a caramel color regent, as the indicator, to a solution, and measured the spin-spin relaxation time with a low-field HNMR technique. In the mixture with caramel solution, the brown shade became increasingly darker as the revolution speed or action number increased. However, in a mixture made with distilled water, the appearance was clear and turbidity reduced as the revolution speed increased. The dark caramel color indicated that the flour had separated into small particles and water was distributed throughout the mixture, meaning that the constituents were well-mixed. However, at action numbers 544 and 680, the mixture began to agglomerate.

Small flour particles containing water were aggregated. In other words, even with continued mixing, the structural change associated with the development of the gluten network was not observed. When flour and water were mixed, a change in color (from bright white to dark) was observed. The colorimeter also highlighted a decreasing trend in the L* value. The relationship between net energy and color parameters indicated that as net energy increased, L* and H decreased, but a* and ΔE increased. The decrease in L* as a function of increasing net energy showed that the caramel solution was well mixed. The authors concluded that it would be interesting and useful to use colorimetric indexes to determine the progress of the kneading process.

6.1.7.3 Use carbonic (CO_2) snow

As reported in section 6.1.6.2, dough warming during kneading significantly worsens rheological properties and bread characteristics (Sadot et al., 2017; Bayramov & Nabiev, 2019; Sangpring et al., 2017; Quayson et al., 2016; Preedy & Watson, 2019). This finding highlights the importance of refrigerating dough during kneading. Cappelli et al. (2020b) evaluated the addition of six percentages (from 0 to 10%) of carbonic snow to dough during mixing, as an alternative refrigerant. The results showed the effectiveness of the technique for dough thermoregulation, and improving bread characteristics. In particular, CO_2 snow was able to rapidly decrease dough temperature in both rheological and breadmaking tests (Cappelli et al., 2020b). While a slight improvement was observed in rheological properties at higher percentages, the technique appears to be most effective when used with closed kneading machines. These observations were confirmed by the final temperature of doughs treated with 6%, 8%, and 10% of CO_2 snow, which were 2 °C, 3 °C, and 4 °C lower than the initial temperature of the sample and the control (Cappelli et al., 2020b).

Furthermore, Cappelli et al. (2020b) found that the positive effects of the proposed strategy were not limited to dough thermoregulation. Beneficial effects were also observed for bread characteristics. In particular, specific volume and loaf height increased. This improvement can be attributed to the effectiveness of this alternative refrigerant in maintaining a low dough temperature during kneading, resulting in improved gluten development (increasing the number of hydrogen bonds) and stabilization of the overall structure (Cappelli et al., 2020b). The authors conclude that high percentages of CO_2 snow (between 6% to 10%) could be used as an alternative refrigerant in the baking industry. Other advantages include ease of application, minimal additional expenditure, higher cooling power (compared to other refrigerants), no increase in total water content, and no chemical or toxic residuals (Cappelli et al., 2020b).

6.1.7.4 Add organic acids

Su et al. (2019) explored the effects of the addition of acetic acid, lactic acid, malic acid, fumaric acid, and citric acid during kneading to prolong the shelf life of loaves, and improve bread characteristics. All organic acids resulted in higher specific volume, lower moisture content, lower pH, and decreased hardness. Specific volume was highest in bread obtained by adding 0.3% citric acid. Moreover, yeast activity was enhanced, although gas retention capability decreased. Organic acids also reduced the molecular weight of proteins and starch. These changes were most significant in dough with 0.3% fumaric acid. Proteolysis and amylolysis mainly occurred after mixing, and depended on the type of acid present in the mixture. The authors concluded that the cleavage of disulfide bonds in gluten might be related to H+ concentrations in the dough system.

Su et al. (2019) argued that organic acids, which are the main metabolites, have two significant functions. The first is to extend shelf life, as the acidic environment they create can improve amylase activity, delay starch retrogradation, and increase the antimicrobial activity of sourdough against saprophytic microorganisms. The second is to improve sensory quality, as the modest proteolysis resulting from their addition can accumulate amino acids that contribute to the formation of flavor substances during baking. Moreover, they can increase elasticity, ductility, and bread specific volume. The authors conclude that the addition of acids, at optimal concentrations, could benefit specific volume by reducing the density and intensity of the gluten network and decreasing resistance when CO_2 is released (Su et al., 2019). However, excessive concentrations might weaken the gluten network and impair gas retention capability.

6.1.7.5 Replace sodium chloride (NaCl) with salt substitutes

Sodium chloride is widely used to improve dough rheological properties, and bread characteristics (Yovchev et al., 2017; Isaak et al., 2019; Sun et al., 2019). Nevertheless, evidence of its negative effects on human health are leading to policies that seek to reduce or eliminate its presence in food products (Amoriello & Carcea, 2019). However, the food industry is not ready to end its use and, consequently, the scientific world is looking for alternatives.

Jekle et al. (2019) investigated the effects of different salt substitutes (CaCl2, MgCl2, LiCl, KCl, NH4Cl) on wheat dough development during mixing and fermentation. Substitutes were measured at the level of 2 g salt/ 100 g wheat flour, with the exception of KCl. The study found that the addition of 2 g of any chloride salt led to a significant decrease in water absorption. The authors argued that this was due to the influence of the altered solvent environment on gluten proteins. Hydration by salt ions leads to changes in gluten molecules, which reduces electrostatic repulsion between molecules (Jekle et al.,2019). Moreover, the study found a significant increase in dough development time with the addition of any chloride salt (compared to salt-free dough). This was thought to be due to delayed protein hydration in the presence of ions, which slowed the development of the protein network (Jekle et al.,2019). The intensity of the slowdown was found to be a function of the chloride salt. The more kosmotropic the salt, the longer is development time, indicating that the development of the gluten network is dependent on the effects of minor components.

Dough stability of salt-free dough was found to be 15.9 ± 0.2 min (Jekle et al.,2019). The addition of 2 g sodium chloride to 100 g wheat flour significantly increased this by 2.7 min (+17%) (Jekle et al.,2019). Significant differences were only found for CaCl2 and NH4Cl. Like dough development time, the more kosmotropic the salt, the greater the impact on stability. The greatest increase was found for NH4Cl (+ 1.64 min) per g salt addition to 100 g flour. These findings could be relevant to the baking industry, where KCl is currently the dominant NaCl substitute.

6.1.7.6 Regulate the addition of water

As reported in section 6.1.6.3, the water absorption of flour and dough water content are fundamental to obtaining dough with good rheological properties and bread with optimal characteristics (Voicu et al., 2017; Cappelli et al., 2018; Cauvain, 2017; Hackenberg et al., 2018a). Nevertheless, the amount of water added during kneading might not be the only parameter to adjust. Yang et al. (2019) investigated whether the modality of water addition during kneading could have positive effects on dough rheology. The authors tested the effect of the addition of the same total amount of water, added at two or three times in the kneading process (7 min total kneading time).

Their study found that the rheological properties of dough made with water added in three stages were better than other samples. The authors argued that, as contact between flour and water is staged, they are fully contacted rather than partially bonded into a cluster, part of which is still dry flour. The technique encourages moisture migration to the inside of the dough, the full formation of the gluten network, and an increase in tensile properties. Their results showed that water holding capacity of dough was enhanced, its water content and tensile properties increased, and polymerization was highest. Furthermore, more water soluble components were incorporated into the water, and lubrication was strengthened, which increased the viscosity and weakened the fluidity of the dough. In conclusion, the correct modality of water addition might be an interesting improvement to the kneading process which need further investigations.

6.1.7.7 Add enzymes

The literature review highlighted various studies of the effects of enzymes on dough rheology and bread characteristics. Redox enzymes (oxidoreductases) are used to directly or indirectly crosslink gluten proteins through various covalent bonds, thus strengthening the dough system. The appropriate use of some endopeptidases, which hydrolyze gluten (to some extent) during mixing and fermentation may also improve bread crumb. The effects of peptidases strongly depend on the kneading method, flour quality and the presence of other functional ingredients (Ooms & Delcour, 2019). Laccase appears to be another interesting enzyme. Tebben et al. (2018) found that it increased dough strength and stability, decreased stickiness, increased loaf volume, and improved crumb structure and softness.

Koksel and Scanlon (2018), investigated the effects of several enzymes (glucose oxidase, xylanase, cellulase, and lipase) on dough rheological properties and bread characteristics. For strong wheat flour doughs, the greatest changes were observed for glucose oxidase, followed by xylanase, and then cellulase. For weak flour doughs, the largest changes were observed for doughs containing lipase and xylanase, with the effect of glucose oxidase being much less pronounced. Moreover, when enzymes were added to weak flour doughs, net aeration during mixing remained the same. The exception was glucose oxidase, where an increase in gas volume fractions compared to the control dough was found. The same study found a strengthening effect of glucose oxidase on dough mechanical properties through the cross-linking of gluten proteins. The authors noted that glucose oxidase, in the presence of oxygen during mixing, promoted the formation of covalent bonds within the gluten network, making the dough stronger.

Furthermore, Koksel and Scanlon (2018) found that lipase modified lipids by catalyzing the hydrolysis of the ester bonds of glycerolipids. With lipase activity, more polar and more reactive lipid compounds are obtained, and the concentration of surface-active materials at the surface of bubbles is likely to increase, modifying the gas phase of the dough. Xylanase activity degrades pentosans to release bonded water, increasing the amount of free water. The dough becomes slack, while the increase in free water content is reflected in a phase velocity depression of ~100 ms, indicating a considerable change in dough matrix properties. The authors concluded that the addition of enzymes to the dough formulation influenced the dough's mechanical properties, the nature and the amount of gas occlusion during mixing, and bread characteristics.

6.1.7.8 Add hydrocolloids

Hydrocolloids improve dough performance, bread characteristics, and sensorial quality. They are also added to minimize undesirable changes in crumb texture during storage (the anti-staling effect). In bakeoff technologies (frozen dough, par-baked bread), they can help to preserve the structure from damage due to freezing (Ferrero, 2017; Tebben et al., 2018). The addition of hydrocolloids to wheat flour changes water absorption due to their hydrophilic nature and, interestingly, the type of hydrocolloid seems to be a more important factor than its concentration (Ferrero, 2017). The latter study found that hydrocolloid competition with gluten proteins for water is a key factor affecting gluten development. However, the effect on dough rheology also depends on specific interactions between hydrocolloids and gluten proteins. The addition of hydrocolloids during kneading has been found to improve dough rheology, bread volume, crumb porosity and texture, leading to products with enhanced technological quality (Ferrero, 2017; Tebben et al., 2018).

These results were confirmed by Farbo et al. (2020), who tested the effects of several hydrocolloids (methylcellulose, guar, psyllium, xanthan, and tara) on the rheological properties of doughs obtained from an old Italian wheat cultivar (Russello). Significant differences were found. In particular, dough extensibility was increased with 1 % psyllium or xanthan gum, while the gas retention coefficient increased with all hydrocolloids. Pasting properties were modified via an increase in final viscosity and the setback value, compared with control semolina (with the exception of methylcellulose); this resulted in a more homogeneous and compact dough structure, and in an improved gluten development.

6.1.7.9 Add emulsifiers

Garzón et al. (2018) tested the effects of several emulsifiers, notably diacetyl tartaric acid ester of monoglycerides, sodium stearoyl lactylate, distilled monoglyceride (DMG-45 and DMG-75), lecithin, and polyglycerol esters of fatty acids (PGEF) on dough rheology and bread characteristics. They found that emulsifiers increased maximum dough volume during proofing, and increased the number of bubbles incorporated during mixing (particularly with PGEF). Emulsifiers appeared to increase the number of smaller gas cells in bread crumb, and increased crumb firmness, suggesting that interactions between emulsifiers and gluten affect protein polymerization during baking. During mixing, emulsifiers increased dough strength and extensibility. In the fermentation stage they improved gas retention and avoided dough collapse, leading to a softer bread crumb, although their effect was found to be closely related to wheat flour protein content and proofing duration.

Garzón et al. (2018) also found that dough stability was closely related to the emulsifier; only lecithin and DMG-45 extended dough stability beyond that of the control. PGEF significantly reduced the longitudinal area, reducing loaf size, while other emulsifiers did not significantly modify this parameter. Although dough volume increased during fermentation, emulsifiers did not appear to increase resistance enough to improve final volume. The authors concluded that emulsifiers behave in different ways because of their diverse chemical structure and physical characteristics. They increase air incorporation into the dough, resulting in a higher number of bubbles (in particular, PGEF).

6.1.7.10 Delay the addition of bran, middlings and germ

The negative effects of bran, middling, and germ addition on dough rheological properties and bread characteristics are widely reported in the literature (Cappelli et al., 2018; Han et al., 2019; Hemdane et al., 2017; Ishwarya et al., 2017; Navrotskyi et al., 2019; Cappelli et al., 2019a; Packkia-Doss et al., 2019). Consequently, Cappelli et al. (2019a) proposed a strategy based on the delayed addition of bran and middlings during kneading. The study assessed differences in dough rheology and bread characteristics as a function of three percentages of bran and middlings content (10–30%) and five addition times (0–6.5 min after kneading begins).

The study found that delayed addition influenced both dough rheology and bread quality. Regarding rheology, the proposed technique reduced tenacity (P), increased extensibility (L) and, consequently, reduced P/L, which is crucial for the technological success of leavened products (Cappelli et al., 2019a). The ameliorative effects on P/L are very interesting, as an increase in this ratio is one of the most problematic defects of whole wheat bread. Beneficial effects were also found for bread characteristics, in particular increased specific volume (Cappelli et al., 2019a). For 10% and 20% whole wheat flour, performance was best for addition at 2 min (25% of total kneading time) (Cappelli et al., 2019a). Although addition at 3.5 min (43.75% of total kneading time) also performed well, W decreased, suggesting that addition at 2 min is more suitable. In the latter case, the dough is kneaded for a further 6 min (compared to 4.5 min with addition at 3.5 min) with beneficial effects on the gluten network (Cappelli et al., 2019a). Finally, no marked effects were found for 30% whole wheat flour, except for bread specific volume. Nevertheless, even in this case, addition at 2 min resulted in highest bread specific volume and best crumb density (Cappelli et al., 2019a).

6.1.8 Strategies to improve kneading machines

6.1.8.1 Vacuum kneading machines

The vacuum processing method has been found to improve dough uniformity and structure, which becomes more compact (Shao et al., 2019). The latter study found an increase in the storage modulus; this indicates that vacuum mixing could enhance dough elasticity and reinforce the network structure of its protein. Moreover, Liu et al. (2020) found that vacuum mixing significantly improved water–solid interactions, resulting in a more continuous and compact structure; it also significantly changed the sulfhydryl content, secondary structure, microstructure, and extensibility of dough. However, its impact has been found to be time-dependent, and this can be used to change the structural and textural quality of doughs (Liu et al., 2020; Liu et al., 2017; Gao et al., 2017a; Gao et al., 2017b).

These results are supported by Gao et al. (2017a), who found a significant increase in dough extensibility when dough was mixed under a moderate vacuum (−0.04 MPa for 3 min) compared to another mixed under atmospheric pressure for the same duration. The authors attributed this finding to the formation of a more extensive gluten network, associated with an increased disulfide bond density and a significantly higher B-sheet to B-turn ratio. The moderate vacuum helped the dough to withstand the longer mixing time, indicated by its increased disulfide bond density and biaxial extensibility compared to the control dough mixed under atmospheric pressure. The authors argue that a significantly reduced headspace pressure promotes the formation of extensive hydrogen bonding in bread dough, regardless of the flour type. The same observation was highlighted by Liu et al. (2017) for an optimal level of vacuum of −0.06. Both Gao et al. (2017a) and Liu et al. (2020) suggest applying a moderate vacuum to reduce mixing time (for high protein flour) and prevent over-mixing (for low protein flour). A higher level of vacuum (−0.08 MPa) was found to be detrimental to dough development, probably because of the limited availability of oxygen (Gao et al., 2017a).

Regarding bread characteristics, Gao et al. (2017b) found that an increased vacuum had a negative impact on low-protein dough mixed for a short duration (3 min), while it had a positive impact when dough was mixed for a longer duration (5 min). This time-dependent effect of vacuum mixing is supported by Liu et al. (2020), who found that a too-high or too-low vacuum, and insufficient or excessive mixing, resulted in decreased resistance to extension of the dough. Compared with the 0 MPa condition, the −0.06 MPa condition increased resistance to extension by 6.9% (Liu et al., 2020), while mixing for 10 min increased it by 16.6% compared to 5 min. The authors noted that when the dough was mixed at −0.06 Mpa, starch granules were tightly embedded in the continuous membrane-shaped gluten matrix, and the starch and gluten structure were tightly combined, mainly because the dough had the highest tightly-bound water content.

Gao et al. (2017b) observed that bread samples made from vacuum mixed high protein flour dough had significantly lower porosity and smaller cell sizes compared to those made from dough mixed under atmospheric pressure. They concluded that vacuum mixing did not adversely affect average yeast activity, but did delay initial gas diffusion. Moreover, vacuum mixing produced bread with a denser crumb structure and harder texture (Gao et al., 2017b). On the other hand, longer mixing, combined with the application of a moderate vacuum during kneading, promoted dough development and resulted in baked and steamed bread with larger volume, more porous structure and softer texture (Gao et al., 2017b).

6.1.8.2 Refrigerate kneading machines

As reported in sections above, it is fundamental to keep the dough temperature low during the kneading phase. Several refrigeration systems have been investigated – examples include coolants, carbonic snow, and cooling water jackets (Sadot et al., 2017; Cappelli et al., 2020b). A temperature-controlled double jacket and an infrared sensor can be used to monitor dough surface temperature (Sadot et al., 2017). An interesting innovation could be to equip mixers with a snow making machine, which could release a specific amount of carbonic snow and regulate dough temperature (Cappelli et al., 2020b). This might increase the usability of the kneading systems, topic which is becoming a key element in the development and design of machines and plants for the food industry (Cappelli et al., 2020i; Cappelli et al., 2019b).

6.1.8.3 Technological improvements

Most studies of mixing parameters, and the optimization of mixing properties examine power and torque using a strain gauge (Jerome et al., 2019; Aljaafreh, 2017; Asaithambi et al., 2020). Although studies of strain gauges are noninvasive and real-time, they only analyze physical and chemical modifications to the dough; notably through the application of near infrared (NIR) spectroscopy, X-ray, and ultrasound-acoustic systems (Jerome et al., 2019). The latter authors highlight that NIR spectroscopy is an effective way to assess both physical and chemical changes because there is a high correlation between measurements derived from NIR mixing curves, and functional and qualitative dough properties calculated from the elastic modulus of flour. X-rays have also been used. A 3D visualization and reconstruction of the internal structure of dough during mixing produced interesting results using X-ray tomography at 10 µm^3, and the same applies to the use of ultrasound-acoustic systems. Although these three technologies appear interesting, they need further investigation.

6.1.8.4 Automatic and adaptive kneading machines

Aljaafreh et al. (2017) propose a novel design for an intelligent process controller, which could automate the kneading process. The design is based on current sensing, and on-line learning through reinforcement using operator input. The system could be a low-cost solution to automating production equipment that is currently operated manually, a situation that is common in the developing world. The approach requires minimal modification to the equipment: a current sensor, an on/ off control relay, button controllers, and an embedded system. During kneading, the change in current load can give valuable information about process performance. A low-cost current sensor can be used monitor to kneading progress, as the machine’s load varies with the dough’s extension resistance. Specifically, changes in dough rheology affect the mixing machine’s torque. This is an interesting, low-cost approach to automating various kinds of production equipment. The future development of automatic and adaptive kneading machines could significantly improve the kneading phase.

6.1.9 Conclusions & future trends

This review has highlighted the influence of the kneading process on dough rheology and bread characteristics. It reveals several critical parameters, notably, kneading time, dough temperature, mixing speed, dough aeration and the addition of ingredients (timing and quantity) (Cappelli et al., 2020a). The key findings can be used to improve all kneading scenarios, at all scales of application. Nevertheless, further steps can be taken to significantly improve the process (Cappelli et al., 2020a). The innovative strategies reported in sections 6.1.7 and 6.1.8 should be carefully weighed as a function of the specific characteristics and aims of the production chain, keeping in mind business needs and product demand (Cappelli et al., 2020a).

For example, if the aim is to improve dough rheology and bread characteristics, it is most important to select the best kneading machine, manage the addition of water, and select the most suitable improver (organic acid, NaCl substitute, enzymes, hydrocolloids, or emulsifiers) (Cappelli et al., 2020a). This should be evaluated on a case-by-case basis, according to the type of product. Nonetheless, it should be noted that all these potential gains will be lost if care is not taken to control the key kneading parameters defined in section 6.1.6 (Cappelli et al., 2020a).

For this reason, we suggest that further improvements will be supported by two developments. First, real-time systems are needed that can measure and control kneading progress, and determine when dough consistency is optimal (the end of the mixing operation) (Cappelli et al., 2020a). Secondly, dough warming must be avoided, and its temperature during kneading must be controlled by the development of better kneading machines and the use of alternative refrigerants such as CO_2 snow (Cappelli et al., 2020a). However, in some cases, other aims are more important. If the aim is to reduce kneading time, prevent overmixing, and minimize production time, vacuum kneading machines, or automatic, adaptive machines might be more useful (Cappelli et al., 2020a).

In conclusion, improvements to the kneading process must begin with a complete understanding of the key parameters (Cappelli et al., 2020a). This should be followed by the implementation of a dedicated, real-time control system that can determine when kneading should end. The final step is to apply the most suitable improvement strategy as a function of production needs (Cappelli et al., 2020a). The logical development of innovations and improvements will have positive effects, not only on dough and bread products, but also on the productivity and profitability of companies, and the entire production chain (Cappelli et al., 2020a).

6.2 Effects of CO_2 snow addition during kneading on thermoregulation, dough rheological properties, and bread characteristics

6.2.1 Aim of the study

As highlighted in earlier work (Cappelli et al., 2020a), dough warming during kneading significantly worsens rheological properties and bread characteristics. Cappelli et al., (2020a; 2020b), Basaran & Göçmen (2003), Rosell & Collar (2009), and Quayson et al. (2016) highlighted the importance of maintaining a low dough temperature during kneading to guarantee the correct development of the gluten network. In particular, Quayson et al. (2016) found that dough rheology and bread performance were closely related to mixing conditions.

The importance of temperature control during the processing of several foods has been reported in the literature, notably for meat, chocolate, butter, and many other processed foods, but it appears that the topic has not been extensively examined in the context of bread and baked products. Given the importance of improvement strategies in the cereal production chain, more consideration should be given to dough thermoregulation and its effects on bread quality, as such research has potential benefits for the food industry, in particular production management during the summer period, which can be characterized by excessive warming of the dough. Furthermore, this technical solution could also result in improvements that are immediately perceptible by consumers (notably, an increase in specific volume for breads made with ancient and weak wheat flours).

Several systems have been employed to refrigerate the dough during kneading – examples include heat exchangers for refrigeration, coolants, and cooling water jackets in machines. However, the food industry is continuously searching for alternative solutions. Consequently, this work evaluates the effectiveness of an alternative refrigerant, namely CO_2 snow. CO_2 snow is a refrigerant that already has applications in areas such as wine production, chicken meat, and frozen food. However, it has not, so far, been used in the domain of dough and the production of baked products, thus motivating this research.

Although the warming of doughs is a problem reported by the baking industry and given the lack of essential studies regarding the effects of CO_2 snow on dough rheology and bread characteristics, we considered important to start with a laboratory-scale experiment with breadmaking carried out with domestic bread machines and not directly with industrial-scale tests. Moreover, the use of bread machines allows standardization of the breadmaking procedure, reducing the errors and differences connected with a straight dough method carried out manually. This is consistent with other authors.

The aim of this research is to examine the effects of the addition of CO_2 snow during kneading on dough temperature and rheological properties, and bread characteristics (Cappelli et al., 2020b). Two type of flour were tested: a modern wheat cultivar (Bologna) and an ancient wheat cultivar (Verna) (Cappelli et al., 2020b). Six percentages of CO_2 snow, based on dry weight, were tested: 0% (control, no CO_2 snow added), 2%, 4%, 6%, 8%, and 10% (Cappelli et al., 2020b).

6.2.2 *Materials and methods*

6.2.2.1 Raw materials and CO_2 snow preparation

Samples of refined white flour (defined 00 under Italian legislation) from modern (Bologna) and ancient (Verna) wheat cultivars were provided by the Paciscopi mill (Montespertoli, Florence, Italy). Tested flours were obtained using an industrial roller mill. The chosen cultivars are representative of Italian bread wheats and are particularly esteemed in the center of Italy. Samples of CO_2 snow were produced and weighed in the laboratory, contextually to each test or replicate, using equipment provided by S.I.A.D. Ltd. The latter equipment consists of pressurized liquid CO_2 tanks connected to a snowmaking machine, which allows the CO_2 to expand and produce solid CO_2 snow. Table 16 shows percentages and weights (in grams) of CO_2 snow added during the alveograph and breadmaking tests, which required 250 g and 310 g of flour, respectively (ISO, 2008). Fresh brewer's yeast (Zeus, Zeus IBA Ltd., Florence, Italy), salt (Chantesel Ltd.), and water (Acqua minerale San Benedetto Ltd.) were purchased in a local supermarket.

Table 16: CO_2 snow addition during alveograph and breadmaking tests. (Source: Cappelli et al., 2020b).

Type of test	Grams of flour	CO_2 snow (%)	CO_2 snow (grams)
Chopin alveograph	250	0	0
Chopin alveograph	250	2	5
Chopin alveograph	250	4	10
Chopin alveograph	250	6	15
Chopin alveograph	250	8	20
Chopin alveograph	250	10	25
Breadmaking	310	0	0
Breadmaking	310	2	6.2
Breadmaking	310	4	12.4
Breadmaking	310	6	18.6
Breadmaking	310	8	24.8
Breadmaking	310	10	31

6.2.2.2 Experimental design

Our experiment aimed to assess differences between tested doughs and breads, as a function of six levels of CO_2 snow content, which were added during kneading both in rheological and breadmaking tests. The tested levels of CO_2 snow content were: 0% (control, no CO_2 snow added), 2%, 4%, 6%, 8%, and 10% of dry weight. Table 16 shows percentages and weights (in grams) of CO_2 snow added during the alveograph and breadmaking tests. As the amount of wheat flour differed in these tests, so did the amount (in grams) of CO_2 snow added. The CO_2 snow was added one minute after the start of the kneading process, both in alveograph and breadmaking tests. This was necessary for two reasons: (1) it enabled the dough to be created (initially it is just a collection of heterogeneous ingredients); and (2) it avoided the potential problem of free water freezing, in samples with high amounts of CO_2 snow.

Following the procedure given in ISO 27971, total kneading time was eight minutes in the Chopin alveograph tests (ISO, 2008), and 20 min in breadmaking tests. A food thermometer equipped with a probe (model Temp 7 Pt 100, XS Instruments, Modena, Italy) was used to measure the evolution of the temperature (accuracy ± 0.1 °C) during kneading. In particular, it was observed and recorded every minute, starting from the initial temperature (T0), then after one minute (T1) when the CO_2 snow was added, and at every subsequent minute until the end of the kneading process.

In the case of Chopin alveograph tests, the implementation of the temperature measurements started with the identification of the initial temperature (T0). Successively, the kneading process was started switching on the kneading machine and adding the predetermined amount of saline solution during the first 40 s of kneading, consistently with ISO 27971 (ISO, 2008). After 1 min of total kneading, the process was stopped, the flour and the dough residuals were scraped from the internal walls of the kneading machine, the carbonic snow was added, and, lastly, the kneading process was restarted for the residual 7 min. This is consistent with ISO 27971 which impose to complete these tasks in maximum 1 min (ISO, 2008). Immediately after this, the probe of the thermometer was inserted through the hole present in the upper door of the kneading machine (the hole through which the saline solution pass in the kneading machine), placing in contact the probe of the thermometer with the dough (inserting the tip of the probe for about two cm), taking care that it did not come into contact with the kneading element of the instrument.

Leaving the tip of the probe inserted in the dough until stable temperature (a few seconds), the temperature of the dough (T1) was measured. Successively, with the same procedure (removing and reinserting the probe), the evolution of the temperature (minute-by-minute) till the end of the kneading process was measured. It is important to highlight that the ISO 27971 (ISO, 2008) allows only one stop of the kneading process and, moreover, it is not possible to remove the upper door of the kneading machine because it is linked to a safety lock which, in case of removal, interrupt the kneading process.

With respect to breadmaking tests, a closeable hole in the upper cover of the bread machines was realized. First was measured the initial temperature (T0). After this, the 20-min kneading process was started. During the first minute of kneading, the exact amount of water (determined according to the water absorption recorded in the farinograph trials (Table 18)) with dissolved the brewer’s yeast, and the salt were added to the flour. Exactly after one minute, without interrupting the kneading process, the carbonic snow was added. By the repeated insertion of the thermometer probe’s inside the closeable hole especially made in the upper cover of the bread machines, the temperature (T1) and all the others temperatures were measured.

In particular, the temperature measurements were performed leaving the tip of the probe inserted in the dough until stable temperature (a few seconds). Like in the Chopin alveograph tests, the tip of the probe was inserted in the dough for about two cm and did not come into contact with the kneading element of the instrument. After each single temperature measurement, the probe was extracted and the hole in the upper cover of the bread machine was closed. The temperature measurement was repeated every minute till the end of the kneading process using the same technique.

6.2.2.3 Flour characterization and analysis

Starch (AOAC 979.10 AOAC International, 2005), protein (AOAC 920.87 AOAC International, 2005), and ash (AOAC 923.03 AOAC International, 2005), were determined by the Analytical Food Laboratory (Florence, Italy) following approved, official methods. Table 17 shows the results of the compositional analysis for the tested flours.

Table 17: Flour characterization and analysis. (Source: Cappelli et al., 2020b).

Sample	Starch (g/100 g)	Protein (g/100 g)	Ash (g/100 g)
Bologna	61.10	10.20	0.4
Verna	57.70	9.60	0.5

6.2.2.4 Rheological properties of doughs

The rheological properties of doughs were evaluated with a Chopin NG alveograph, linked to an alveolink integrator–recorder (Chopin technologies, Villeneuve-La-Garenne, France). A new sample of CO_2 snow was produced for each test, weighed, and then added into the instrument’s kneading machine, exactly one minute after kneading began. The evolution of dough temperature, before and after the addition of CO_2 snow, was monitored and recorded as described above, throughout the eight minutes of the kneading process (ISO, 2008). Dough tenacity (P), dough extensibility (L), deformation energy (W), the index of swelling (G), and the curve configuration ratio (P/L) were evaluated.

Furthermore, and consistent with other authors (Parenti et al., 2020), differences in dough rheological properties between Bologna and Verna cultivars were examined in more detail. Specifically, farinograph tests (three replicates) were used to determine optimal breadmaking conditions (Brabender, Duisburg, Germany). As described in the method ICC 115/1 of the International Association for Cereal Science and Technology (International Association for Cereal Chemistry, 1992), water absorption (WA), dough development time (DDT, the time to reach maximum consistency in minutes), dough stability (S, the time for which dough consistency remains at 500 Brabender Units), degree of softening (DS, the consistency difference between height at peak and that 5 min later in Brabender Units), and the twenty minute drop (TMD, the difference in Brabender Units from the 500 line to the center of the curve measured at 20 min) were assessed.

6.2.2.5 Breadmaking process

With respect to the breadmaking procedure, the straight dough method was applied. Mixing of the ingredients, dough formation, resting, leavening with fresh brewer's yeast, and baking, were performed using a domestic bread machine (Pain Dorè, Moulinex, Ecully, France). The following recipe was used: 310 g of wheat flour, 13 g of brewer's yeast, 9 g of salt, and a variable amount of water according to the water absorption percentages recorded in farinograph trials (Table 18). The optimum amounts of water added were 54.20% for Bologna and 48.70% for Verna, respectively. The kneading process was carried out at 110 RPM and 20 °C for 20 min. The CO_2 snow was added exactly one minute after the kneading process began. Fermentation and proofing were performed at 40 °C for 1 h and 33 min. Finally, baking was carried out at 180 °C for 48 min. After baking, breads were cooled to room temperature and stored in paper bags, following current practice.

6.2.2.6 Bread characterization

Bread specific volume (L/kg) was evaluated using the standard millet displacement method (AACC, 2000), in accordance with other authors and earlier works (Parenti et al., 2020; Cappelli et al., 2019a). Bread loaf height (mm) was measured with a caliper at the center of the loaf. Crumb density (g/ml) was determined from the mass/volume ratio. Crumb and crust moisture (g/100 g) were measured by gravimetry at 105 °C until a constant weight was reached.

6.2.2.7 Statistical analysis

The effects of CO_2 snow addition during kneading were assessed using a one-way ANOVA, which was applied to each alveographic and bread parameter to establish its significance. Six levels were tested: 0% (control, no CO_2 snow added), 2%, 4%, 6%, 8%, and 10%. Significance was set at $p<0.05$. In cases of statistically significant results ($p < 0.05$) a Tukey HSD post hoc test was performed.

6.2.3 Results and discussion

6.2.3.1 Evolution of dough temperature during Chopin alveograph tests

The evolution of the dough temperature during the kneading process in the alveograph tests is shown in Fig. 13 (Bologna) and Fig. 14 (Verna).

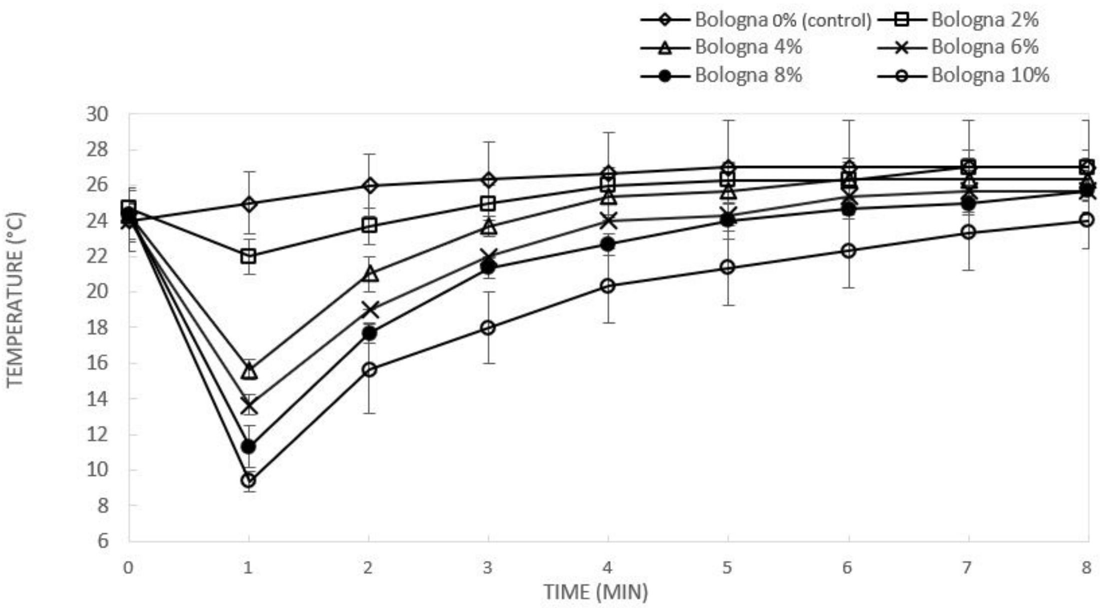

Fig 13: Temperatures of dough made with Bologna flour during the kneading process in Chopin alveograph tests. Data points represent the mean of the three replicates ± SD. (Source: Cappelli et al., 2020b).

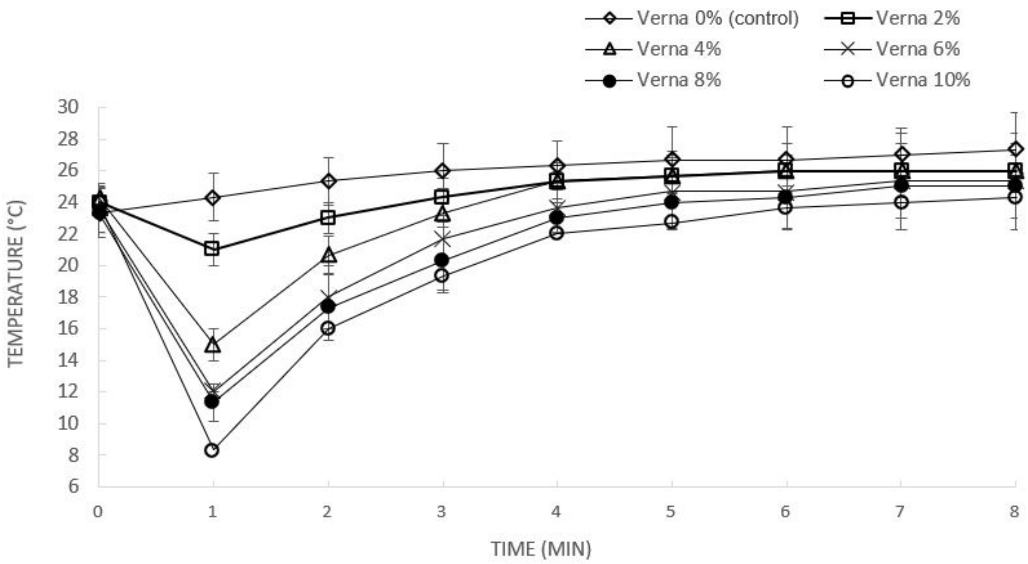

Fig 14: temperatures of dough made with Verna flour during the kneading process in Chopin alveograph tests. Data points represent the mean of the three replicates ± SD. (Source: Cappelli et al., 2020b).

Regarding doughs made with Bologna flour, the final temperature of the control dough was 3 °C higher (27.0 ± 2.6) than the initial temperature (24.0 ± 1.7). The addition of CO_2 snow decreased dough temperature at the moment of the addition (1 min.). In particular, the addition of 2%, 4%, 6%, 8%, and 10% of CO_2 snow, resulted in a respective decrease of 2.7 °C, 8.6 °C, 10.3 °C, 13 °C, and 15 °C, compared to the initial temperature (Fig. 13). The biggest falls were found with the highest percentages of CO_2 snow addition (i.e. 6%, 8%, and 10%). Nevertheless, as the kneading machine of the Chopin alveograph is an open system that can exchange heat with the external environment, the dough temperature quickly and abruptly rose during the following seven minutes of kneading (Fig. 13). In fact, only the temperature of the dough with 10% CO_2 snow addition (24.0 ± 1.5) was comparable to the initial temperature of the control (24.0 ± 1.7). Nevertheless, it seems that in the case of the highest percentages of CO_2 snow addition, a slightly lower variability of temperatures is observable. This might be due to the higher cooling power in the case of higher percentages of CO_2 snow, which allow to obtain, in less time, a more uniform temperature in the mass and to reduces the edge effect, leading to more stable temperatures. At the same time, all doughs made with CO_2 snow had a final temperature that was lower than the final temperature of the control.

With respect to doughs produced with Verna flour, the final temperature of the control dough was 4 °C higher (27.3 ± 2.3) than the initial temperature (23.3 ± 1.5). As observed for Bologna samples, the addition of CO_2 snow decreased dough temperature (Fig. 14). In particular, the addition of 2%, 4%, 6%, 8%, and 10% CO_2 snow, resulted in a respective drop of 3 °C, 9.3 °C, 11 °C, 12 °C, and 15.7 °C, compared to the initial temperature (Fig. 14). The biggest falls were reached with the highest percentages of CO_2 snow (i.e. 6%, 8%, and 10%). Here again, although the dough temperature quickly and abruptly rose during the successive seven minutes of kneading (Fig. 14), the final temperature was lower in test samples compared to the control. Finally, as observed for Bologna samples, in the case of the highest percentages of CO_2 snow addition a lower variability of temperatures, due to the same reason described above, is observable.

6.2.3.2 Evolution of dough temperature during breadmaking

The evolution of dough temperature during breadmaking is shown in Fig. 15 (Bologna) and Fig. 16 (Verna).

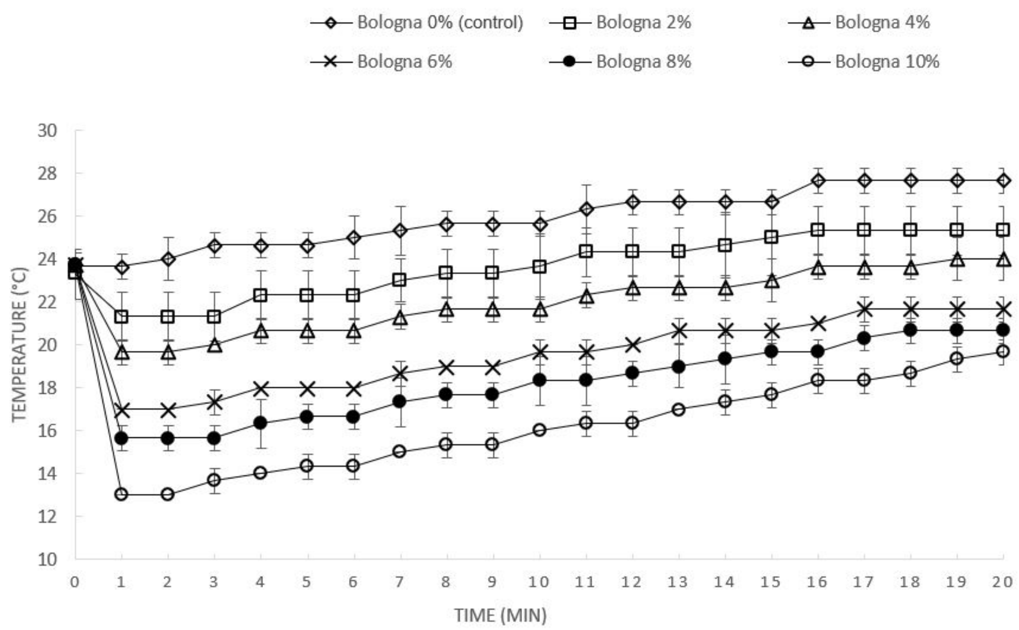

Fig 15: Temperatures of dough made with Bologna flour during the kneading process in breadmaking tests. Data points represent the mean of the three replicates ± SD. (Source: Cappelli et al., 2020b).

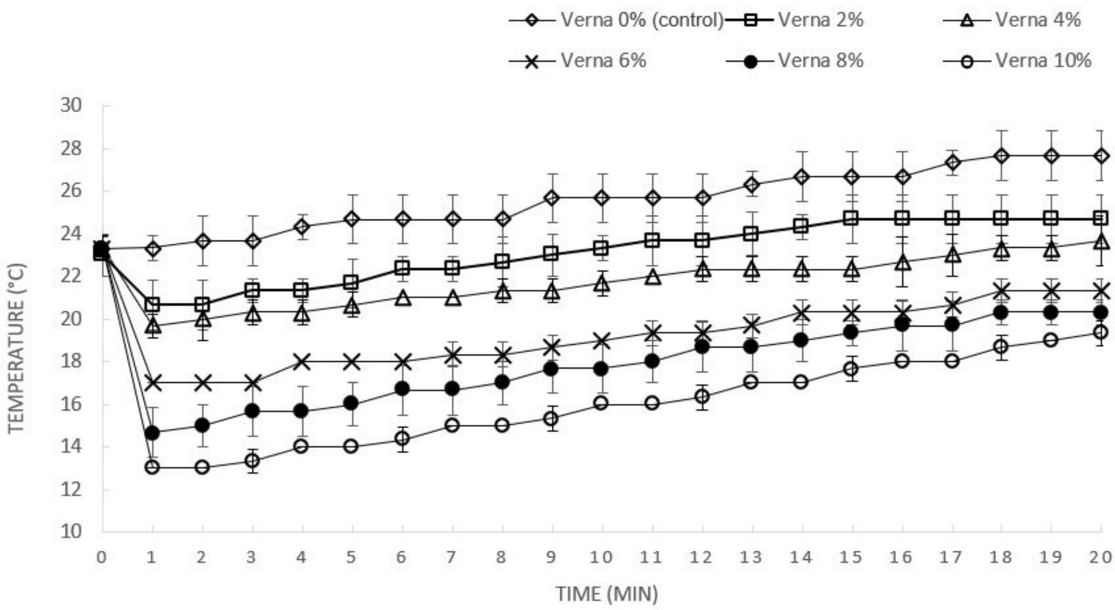

Fig 16: Temperatures of dough made with Verna flour during the kneading process in breadmaking tests. Data points represent the mean of the three replicates ± SD. (Source: Cappelli et al., 2020b).

Regarding doughs produced with Bologna flour, at the end of the 20-minute kneading process, the final temperature of the control dough was 4 °C higher (27.7 ± 0.6) than the initial temperature (23.7 ± 0.6). As observed in alveograph tests, the addition of CO_2 snow decreased dough temperature at the moment of the addition (1 min.) (Fig. 15). Specifically, the addition of 2%, 4%, 6%, 8%, and 10% of CO_2 snow, generated a respective decrease of 2 °C, 4 °C, 6.7 °C, 8 °C, and 10.7 °C, with respect to the initial temperature (Fig. 15).

However, unlike the abrupt temperature change observed in alveograph tests, dough temperature rose more slowly and progressively during kneading (Fig. 15). This is due to differences in the kneading systems used in the Chopin alveograph and the bread machine. Bread machines, unlike the kneading machine of the Chopin alveograph, are closed (but not adiabatic) systems that allow limited exchange with the external environment. This is confirmed by the final temperature of tested doughs, which were, for all tested percentages, lower than the final temperature of the control (Fig. 15). In particular, in the case of the addition of 6%, 8%, and 10% CO_2 snow, final dough temperatures were, respectively, lower by 2 °C, 3 °C, and 4 °C, compared to the initial temperature of the sample and the control (Fig. 15). Moreover, consistently with the results of Chopin alveograph trials, it seems that when the highest percentages of CO_2 snow were added, a decrease in the variability of temperatures was reached. This may be related to the obtaining of a more uniform temperature in the mass and to the reduction of the edge effect generated by the higher cooling power in the case of higher percentages of CO_2 snow, which lead to more stable temperatures.

Regarding doughs made with Verna flour, the final temperature of the control was 4.4 °C higher (27.7 ± 1.2) than its initial temperature (23.3 ± 0.6). Consistently with the findings reported above, the addition of CO_2 snow decreased dough temperature (Fig. 16). In particular, the addition of 2%, 4%, 6%, 8%, and 10% of CO_2 snow led to respective decreases of 2.3 °C, 3.5 °C, 6.3 °C, 8.6 °C, and 10.3 °C, compared to the initial temperature (Fig. 16). As observed for Bologna flour (Fig. 15), dough temperature rose less during breadmaking (Fig. 16), despite a shorter kneading process used in the Chopin alveograph (8 min). Here again, the increase in temperature was slower and more progressive (Fig. 16), for the reasons given above (i.e. differences in the kneading machines). Consistently, the final temperature of the samples additioned with CO_2 snow were lower than the final temperature of the control (Fig. 16): the results showed reductions of 2 °C, 3 °C, and 4 °C, in the case of the addition of 6%, 8%, and 10% of CO_2 snow, compared to the initial temperature of the sample and the control (Fig. 16). Finally, as observed for Bologna samples, when the highest percentages of CO_2 snow were added, a decrease in the variability of temperatures was reached, consistently with the reason described above.

6.2.3.3 Dough rheological tests

The results of farinograph tests are reported in Table 18.

Table 18: Results of farinograph tests expressed as the mean of the three replicates ± SD. (Source: Cappelli et al., 2020b).

Sample	Water absorption (WA)	Dough development time (DDT)	Dough stability (S)	Degree of softening (DS)	Twenty minute drop (TMD)
Bologna	54.2 ± 0.40	3.00 ± 0.50	10.67 ± 2.52	53.33 ± 5.77	83.33 ± 15
Verna	48.7 ± 0.25	1.83 ± 0.29	4.08 ± 0.14	120 ± 20	190 ± 26

The aims of these trials was to determine the optimum amounts of water to be added to Bologna and Verna flours during breadmaking and, moreover, to highlight differences in the rheological properties of the tested cultivars. Regarding water absorption (WA), this was higher for Bologna flour than Verna flour (Table 18), due to its higher protein and starch content (Table 17) (Ma et al., 2007; Cappelli et al., 2020f; Kucek et al., 2017). Moreover, Bologna flour also had higher dough development time (DDT) and higher dough stability (S) (Table 18). This is related to its higher protein content (Table 17) and to the stronger gluten network in modern cultivars. The latter is due to a lower gliadins/ glutenin ratio (i.e. higher glutenin content), improved glutenin allelic composition (due to the introduction of high-quality alleles at the Glu-B1 and Glu- B3 loci), and the differential expression of specific storage proteins (De Santis et al., 2017; Cappelli et al., 2020b). The superior performance of Bologna flour compared to Verna flour was confirmed by the results of the degree of softening (DS) and twenty minute drop (TMD) tests, which both investigated dough weakening after 5 and 20 min. Table 18 shows the lower values of DS and TMD for the Bologna cultivar.

With respect to the effects of CO_2 snow addition on dough rheological properties, Table 19 summarizes the results of the Chopin alveograph tests.

Table 19: Results of alveograph tests (mean of five measurements (diskettes) for each proof) expressed as the mean of the three replicates ± SD. Columns without letters were not significantly different. (Source: Cappelli et al., 2020b).

Sample	P (Dough tenacity)	L (Dough extensibility)	G (Index of swelling)	W (Deformation energy)	P/L
Bologna 0% (control)	114.47 ± 11.05	40.73 ± 10.83	14.11 ± 1.83	195.40 ± 29.30	3.00 ± 0.87
Bologna 2%	112.93 ± 11.94	38.33 ± 2.77	13.77 ± 0.48	193.47 ± 18.25	2.98 ± 0.39
Bologna 4%	113.40 ± 9.30	37.53 ± 5.46	13.61 ± 0.98	187.93 ± 29.41	3.08 ± 0.42
Bologna 6%	111.47 ± 6.80	37.80 ± 4.81	13.67 ± 0.86	184.93 ± 20.04	3.01 ± 0.46
Bologna 8%	113.80 ± 8.14	38.60 ± 3.62	13.11 ± 0.66	184.07 ± 17.57	3.13 ± 0.56
Bologna 10%	113.80 ± 9.26	39.20 ± 0.87	13.93 ± 0.15	195.20 ± 15.46	2.93 ± 0.21
Verna 0% (control)	26.40 ± 1.74	29.20 ± 3.98	12.02 ± 0.81	26.53 ± 4.00	0.92 ± 0.09
Verna 2%	28.87 ± 2.84	30.07 ± 0.50	12.21 ± 0.09	29.47 ± 3.11	0.97 ± 0.09
Verna 4%	28.07 ± 1.81	30.60 ± 2.62	12.30 ± 0.52	28.67 ± 1.55	0.94 ± 0.13
Verna 6%	30.93 ± 4.12	33.53 ± 6.29	12.85 ± 1.17	33.27 ± 7.83	0.95 ± 0.10
Verna 8%	30.47 ± 2.05	33.33 ± 5.43	12.79 ± 0.98	31.67 ± 5.26	0.92 ± 0.12
Verna 10%	29.80 ± 3.47	33.93 ± 4.69	12.93 ± 0.90	32.07 ± 1.92	0.91 ± 0.21

The results of the ANOVA did not find any statistically significant differences for any of the tested alveograph parameters, either for Bologna or Verna cultivars. For both cultivars, the values of alveograph parameters remained substantially stable (Table 19). However, regarding Verna flour, the addition of higher percentages of CO_2 snow slightly increased dough tenacity (P), dough extensibility (L), and deformation energy (W) (Table 19). Although the ANOVA did not highlight any statistically significant results, a modest change in dough rheological properties in the case of the addition of 6%, 8%, and 10% CO_2 snow was observed. The lack of statistically significant differences in the alveograph tests might be related to two issues: first, in Chopin alveograph tests, the temperature remains low for a very short time (Figs. 13 and 14). Maintaining a low dough temperature during kneading has positive effects on gluten development (Cappelli et al., 2020a and 2020b; Basaran and Göçmen, 2003; Rosell and Collar, 2009; Quayson et al., 2016), and the short kneading period may have led to the absence of a significant improvement. Second, the abrupt fall and rise of temperature during this short kneading time (8 min) is not beneficial for the development of gluten (Figs. 13 and 14).

6.2.3.4 Bread characteristics

Table 20 summarizes the results of the effects of CO_2 snow addition on bread specific volume, crumb density, loaf height, and crumb and crust moisture.

Table 20: Results of bread characterization and p-values assessed with the one-way ANOVA. Results are expressed as the mean of the three replicates ± SD. (–) indicates no significant difference at p < 0.05. Columns without letters were not significantly different. (Source: Cappelli et al., 2020b)

Sample	Bread specific volume (L/kg)	Crumb density (g/ml)	Loaf height (mm)	Crumb moisture (%)	Crust moisture (%)
Bologna 0% (control)	2.47 ± 0.05^{a}	0.361 ± 0.03	76.37 ± 2.49^{a}	42.87 ± 0.23	24.84 ± 0.51
Bologna 2%	2.56 ± 0.10^{ab}	0.342 ± 0.01	79.70 ± 2.87^{ab}	42.69 ± 0.18	24.92 ± 1.62
Bologna 4%	2.63 ± 0.15^{ab}	0.353 ± 0.03	81.10 ± 3.32^{b}	42.94 ± 0.58	25.52 ± 1.36
Bologna 6%	2.71 ± 0.06^{b}	0.344 ± 0.04	84.90 ± 2.31^{bc}	42.87 ± 0.33	25.52 ± 1.64
Bologna 8%	2.72 ± 0.13^{b}	0.315 ± 0.01	87.47 ± 1.34^{c}	42.60 ± 0.51	25.50 ± 0.56
Bologna 10%	2.73 ± 0.09^{b}	0.328 ± 0.01	87.07 ± 2.00^{c}	42.71 ± 0.17	24.17 ± 0.85
Verna 0% (control)	2.48 ± 0.13^{a}	0.351 ± 0.05	64.87 ± 0.46^{a}	39.98 ± 1.39	25.47 ± 2.73
Verna 2%	2.52 ± 0.20^{a}	0.374 ± 0.06	69.90 ± 1.39^{b}	40.72 ± 0.85	25.29 ± 0.79
Verna 4%	2.70 ± 0.17^{ab}	0.355 ± 0.03	71.03 ± 0.29^{bc}	40.97 ± 0.61	24.46 ± 0.74
Verna 6%	2.78 ± 0.17^{b}	0.320 ± 0.03	71.93 ± 0.91^{bc}	40.49 ± 0.82	24.09 ± 1.13
Verna 8%	2.88 ± 0.10^{b}	0.333 ± 0.03	72.27 ± 0.87^{c}	40.35 ± 0.48	23.66 ± 1.17
Verna 10%	2.89 ± 0.10^{b}	0.335 ± 0.04	73.20 ± 1.44^{c}	40.42 ± 0.52	24.05 ± 1.18
Factor "percentage of CO_2 snow"		**Significance**			
Bologna	$p < 0.046$	–	$p < 0.001$	–	–
Verna	$p < 0.020$	–	$p < 0.001$	–	–

- Bread specific volume

Regarding bread specific volume, the one-way ANOVA found a significant main effect for the factor "percentage of CO_2 snow added during kneading", both for Bologna and Verna flours (Table 20). In particular, for Bologna flour, specific volume obtained with the addition of 6% (p 0.013), 8% (p 0.010), and 10% (p 0.010) were significantly higher compared to the control. Results were also significant for 6% (p 0.034), 8% (p 0.007), and 10% (p 0.006) Verna flour samples. Table 20 shows a clear trend of increasing specific volume with the increase in CO_2 snow percentage. This observation might be related to two phenomena. First, maintaining a low dough temperature during kneading improves gluten development (Cappelli et al., 2020a and 2020b; Basaran and Göçmen, 2003; Rosell and Collar, 2009).

In particular, low temperatures strengthen and increase hydrogen bonds (Brandner et al., 2019; Quayson et al., 2016; Kontogiorgos and Kasapis, 2010). Although these are weaker than disulfide bonds, they are present in higher numbers and play a key role in the stabilization and strengthening of the structure. At the same time, the low temperature promotes the formation of macromolecular aggregates (Quayson et al., 2016; Kontogiorgos and Kasapis, 2010). Second, the sublimation of CO_2 snow creates an environment with reduced oxygen, which supports yeast fermentation; in fact, CO_2 snow and dry ice are widely used in red wine production to improve the vinification process (Aleixandre-Tudo and Du Toit, 2018).

- Loaf height

The results of the one-way ANOVA for loaf height found a significant main effect of the factor "percentage of CO_2 snow added during kneading", both for Bologna and Verna flours (Table 20). Like specific volume, Table 20 shows that it increased as the percentage of CO_2 snow increased. For Verna flour, all tested percentages of CO_2 snow produced bread with significantly higher heights than the control (p <0.001). With respect to Bologna flour, only the addition of 4% (p 0.037), 6% (p 0.001), 8% (p<0.001), and 10% (p<0.001) CO_2 snow were significant. The reasons for this are the same as for bread specific volume, as volume and loaf height are closely-related parameters.

- Crumb density

With respect to crumb density, the one-way ANOVA did not find any statistically significant differences (Table 20). The reduction of the dough temperature was not able to significantly reduce the crumb density, which seems to be influenced by others factors like nitrogen fertilization of wheat (Guerrini et al., 2020), protein content of flour (Guerrini et al., 2020), and the correct management of water, bran, and middlings in breadmaking (Zanoletti et al., 2017).

- Crumb and crust moisture

Concerning crumb and crust moisture, the one-way ANOVA did not find any statistically significant differences (Table 20). These results should be considered as positive, as they highlight the high degree of purity of the product. Furthermore, unlike other refrigerants, such as ice, which increase water content in doughs and breads, our results highlight the effectiveness of CO_2 snow in dough thermoregulation and, moreover, in the improvement of the most important bread parameters (i.e. volume and loaf height).

6.2.4 Conclusions

The results presented here show the effectiveness of CO_2 snow addition in dough thermoregulation, and in the improvement of bread characteristics (Cappelli et al., 2020b). In particular, it was able to rapidly decrease dough temperature in both rheological and breadmaking tests (Cappelli et al., 2020b). The cooling effect and the ability to maintain a low temperature during kneading appears to be more effective during breadmaking in closed kneading machines (Cappelli et al., 2020b).

This is confirmed by the final temperature of the doughs additioned with 6%, 8%, and 10% of CO_2 snow, which were 2 °C, 3 °C, and 4 °C lower than the initial temperature of the sample and the control, both for Verna and Bologna flours (Cappelli et al., 2020b). Furthermore, the positive effects of the proposed strategy are not limited to dough thermoregulation – beneficial effects are also observed for bread characteristics, notably, increased specific volume and loaf height (Cappelli et al., 2020b).

This improvement can be attributed to the effectiveness of this alternative refrigerant in maintaining a low dough temperature during kneading, resulting in improved gluten development (increasing the number of hydrogen bonds) and stabilization of the overall structure (Basaran and Göçmen, 2003; Rosell and Collar, 2009; Brandner et al., 2019; Quayson et al., 2016; Kontogiorgos and Kasapis, 2010; Cappelli et al., 2020a and 2020b). Moreover, CO_2 snow sublimation supports yeast fermentation (Aleixandre-Tudo and Du Toit, 2018). Ultimately, we recommend the use of high percentages of CO_2 snow (i.e. 6%, 8%, and 10%) as an alternative refrigerant in the baking industry (Cappelli et al., 2020b).

This laboratory-scale experiment provides a first study regarding the application of CO_2 snow in bakery products, which could guide industrial-scale applications. The development of innovative kneading machines with an integrated snow-maker to automatically produce and add CO_2 snow during dough kneading could be possible. Despite the interesting results obtained in this work, further studies are necessary, particularly regarding industrial-scale applications.

In conclusion, our study shows the effectiveness of CO_2 snow in dough thermoregulation and in the improvement of key bread quality parameters, such as specific volume and loaf height. Other advantages include its ease of application, higher cooling power (compared to other refrigerants), no increase in total water content, and no chemical or toxic residuals.

6.3 *Improving whole wheat dough tenacity and extensibility: A new kneading process*

6.3.1 *Aim of the study*

Whole wheat flour (WWF) has become particularly important in the bakery industry due to the higher levels of dietary fiber, vitamins, minerals, antioxidants, and other essential micronutrients found in whole wheat bread (WWB) (Tebben et al., 2018). Whole grain intake has been related to various health benefits and the reduction of several chronic diseases including diabetes, obesity, cancer and cardiovascular diseases (Tebben et al., 2018). There is growing consumer interest in bakery products made with unrefined flours, by virtue of their capability to provide health benefits through bioactive compounds. This increased consumer attention to the health benefits of foods has led to the revival of ancient grains, with beneficial effects for biodiversity (Dinelli et al., 2009; De Santis et al., 2017) and the environment (Recchia et al., 2019). However, the downside is that, despite their improved nutritional profile (Dinelli et al., 2009), the rheological and technological properties of ancient wheats flours perform less well than modern cultivars (De Santis et al., 2017); this becomes even more evident when WWF is used for breadmaking (Cappelli et al., 2018).

Two practices are currently used to produce WWF: either the wheat is milled (predominantly with a stone mill), and stored or used; or it is milled (typically with a roller mill) and bran and middlings (B&M) are successively reinserted to the refined white flour. Although the former, in particular, stone-milled WWF made from ancient wheats, is popular among consumers its functionality and nutritional value decrease rapidly during storage due to the activity of enzymes present in bran and germ fractions that are the origin of lipolytic rancidity. The latter product is seen by some consumers as a “non-natural” product, reducing its popularity (Guerrini et al., 2020; Cappelli et al., 2018). Nevertheless, the separation of refined white flour from B&M, which are successively reinserted, has significant advantages related to storage and is therefore easier for millers to manage.

The use of WWF in bakery products incurs significant rheological problems, primarily linked to the addition of non-endosperm components of the caryopsis (i.e. B&M) (Boita et al., 2016). The effect is due to the negative influence of arabinoxylans, inulin, and β-glucans contained in B&M on the development of the gluten network (Boita et al., 2016; Courtin and Delcour, 2002; Arufe et al., 2017). Arabinoxylans, in particular, affect dough rheology and bread characteristics by binding water, increasing viscosity and disturbing the formation of the protein network during development (Pavlovich-Abril et al., 2016). The most noteworthy rheological problems associated with B&M addition are an increase in tenacity (P) (Cappelli et al., 2018) and viscosity (Le Bleis et al., 2015), a decrease in extensibility (L) (Garofalo et al., 2011), a decrease in dough strength (Gomez et al., 2011) and finally, a significant rise in the curve configuration ratio (i.e. the ratio between P and L) (Cappelli et al., 2018). Furthermore, the bread produced with WWF is less performant than refined flours (Zanoletti et al., 2017). In particular, volume is reduced (Tebben et al., 2018) and crumb density and crumb moisture increased (Zanoletti et al., 2017).

Consequently, strategies to limit the negative effects of adding B&M to whole wheat dough (WWD) are indispensable. Many authors have studied WWD rheology, and proposed ways to improve both dough rheology and bread characteristics. Of these, the most interesting relate to the correct dosage of B&M (Packkia-Doss et al., 2019), dough water content (Cappelli et al., 2018), the addition of gelatinized flours (Parenti et al., 2019) and the use of enzymes such as xylanase, amylases, glucose oxidase, and phytase (Tebben et al., 2018). However, few studies have examined strategies that aim to improve a crucial step, namely the kneading process. This paper addresses the gap. Given that the addition of B&M is essential for the manufacture of WWD and WWB but, at the same time, is the primary cause of poorer rheological and technological performance, this paper examines the effects of delaying the addition of non-endosperm components during kneading. The aim is to improve dough rheological parameters and bread characteristics. In particular, we seek to verify whether this modified procedure can enhance gluten development and improve WWB characteristics, both in technological and nutritional terms.

6.3.2 *Materials and methods*

6.3.2.1 Raw materials and flour preparation

Refined white flour and B&M (Verna cultivar) were kindly provided by New.co.pan Ltd. (Montaione, Florence, Italy) and sieved at 140 μm, 500 μm, and 950 μm, respectively. The choosed cultivar is representative of Italian bread wheats and it is particularly relevant in Tuscany. The milling process was performed by the company who furnished the flour with an industrial roller mill. The milling extraction rates were: refined white flour 74.35%, middlings 8.42%, and bran 17.23%. Three WWF were tested, containing 10%, 20%, and 30% B&M, consistent with the proportions usually found in the milling process. Table 21 shows percentages and weights (in grams) of refined flour and B&M. Table 21a provides details of the preparation of the three WWF used in the Chopin alveograph assessment of rheological properties, which utilize 250 g of flour (ISO 27971 International Standard, 2008). Table 21b concerns the preparation of the three WWF used in the breadmaking procedure with commercial bread machines (Pain Dorè, Moulinex, Ecully, France). Salt (Chantesel Ltd.), brewer's yeast (Zeus, Zeus IBA Ltd., Florence, Italy) and water (Sant'Anna, Fonti di Vinadio Ltd.) were purchased in a local supermarket.

Table 21: Percentages and weight (grams) of refined flour, middlings and bran used for the preparation of the tested whole wheat flours. 1a) Assessment of rheological properties with the Chopin alveograph, with 250 g flour. 1b) Preparation of the three flours tested in the breadmaking procedure, which used 310 g flour according to the standard recipe. (Source: Cappelli et al., 2019a).

Sample	Refined flour (%)	Middlings (%)	Bran (%)	Refined flour (g)	Middlings (g)	Bran (g)
a) Chopin alveograph test						
Whole wheat flour 10%	90	3.28	6.72	225	8.21	16.79
Whole wheat flour 20%	80	6.57	13.43	200	16.42	33.58
Whole wheat flour 30%	70	9.85	20.15	175	24.62	50.38
b) Breadmaking						
Whole wheat flour 10%	90	3.28	6.72	279	10.18	20.82
Whole wheat flour 20%	80	6.57	13.43	248	20.36	41.64
Whole wheat flour 30%	70	9.85	20.15	217	30.53	62.47

6.3.2.2 Experimental design

Our experiment assessed differences between the tested doughs and breads as function of three levels of B&M content (10%, 20%, and 30%) and five B&M addition times during kneading: 0 min (i.e. the WWF already contained B&M (control condition)), 2 min, 3.5 min, 5 min, and 6.5 min. It is important to highlight that these five times correspond respectively to 0%, 25%, 43.75%, 62.50%, and 81.25% of total kneading time (Cappelli et al., 2019a). A full factorial experimental design evaluated variation in rheological properties and bread characteristics (Cappelli et al., 2019a). All tests were carried out in three replicates.

6.3.2.3 Rheological properties of dough

Rheological properties were evaluated with Chopin NG alveograph, linked to an alveolink integrator–recorder (Chopin technologies, Villeneuve-La-Garenne, France) (Cappelli et al., 2019a). B&M were first homogenized and then added into the kneading machine of the instrument, at the respective addition times, without interrupting the kneading process. The alveograph procedure was slightly modified to assess the effects of the delayed addition of B&M on the rheological properties of doughs. This entail a variable amount of refined flour (i.e. 225, 200, and 175 g for whole wheat flour at 10, 20, and 30% respectively) to which was added the sodium chloride solution and, at the five times reported above, B&M to reach the amount of 250 g consistent with the ISO 27971 (ISO, 2008).

The kneading process itself took eight minutes, following the standard protocol (ISO, 2008). Dough tenacity (P), dough extensibility (L), deformation energy (W), the curve configuration ratio (P/L) and the index of swelling (G) were evaluated. Furthermore, to examine WWD rheology in more detail, and determine optimal breadmaking conditions, farinograph tests (Brabender, Duisburg, Germany) were carried out on the three WWF samples. As stated in the International Association for Cereal Science and Technology method (ICC 115/1 (International Association for Cereal Chemistry, 1992)), water absorption (WA), dough development time (DDT, time to reach maximum consistency in minutes), dough stability (S, time in which dough consistency remains at 500 Brabender Units) and the twenty minute drop (TMD, the difference in Brabender Units from the 500 line to the center of the curve measured at 20 min) were measured for the three replicates.

6.3.2.4 Breadmaking process

The breadmaking procedure followed the straight dough method. A bread machine (Pain Dorè, Moulinex, Ecully, France) was used to mix the ingredients, followed by dough formation, resting, leavening with fresh brewer's yeast (Zeus, Zeus IBA Ltd., Florence, Italy), and baking. The mixing protocol was divided into three steps. First, 310 g of flour were mixed at 20 °C and 110 rpm for 3 min, in order to homogenize the sample. Secondly, 9 g of salt and water were mixed with 13 g of fresh brewer's yeast in order to start the kneading process, which ran for 8 min at 20 °C and 110 rpm.

The optimum amount of water to be added was determined by a WWF farinograph (Brabender, Duisburg, Germany) at 10%, 20% and 30%. This determined that optimum amounts were 55.50%, 59.10% and 63.00% for WWF at 10%, 20% and 30% respectively. Thirdly, B&M were weighed (Table 21), mixed and added at the five times reported above. Fermentation and proofing were performed at 40 °C for 1 h and 21 min. Finally, baking was carried out at 180 °C for 55 min. After baking, breads were cooled to room temperature and stored in paper bags, following current practice.

6.3.2.5 Bread characterization

Bread volume (L) was evaluated using the standard millet displacement method (AACC, 2000), as reported by other authors (Parenti et al., 2019; Cappelli et al., 2019a). Crumb density (g/ml) was determined from the mass/volume ratio, in accordance with other authors (Le Bleis et al., 2015; Cappelli et al., 2019a). Crumb and crust moisture (g/100 g) were measured by gravimetry at 105 °C until a constant weight was reached.

6.3.2.6 Flour characterization and analysis

Protein (AOAC 920.87 (AOAC International, 2005)), starch (AOAC 979.10 (AOAC International, 2005)), insoluble fiber (AOAC 991.43 (AOAC International, 2005)), soluble fiber (AOAC 991.43 (AOAC International, 2005)), and ash (AOAC 923.03 (AOAC International, 2005)), were determined by the Analytical Food Laboratory (Florence, Italy) following approved, official methods. Table 22 shows the composition of the tested WWF.

Table 22: Flour characterization and analysis. (source: Cappelli et al., 2019a).

Sample	Protein (g/ 100 g)	Starch (g/ 100 g)	Soluble fiber (g/ 100 g)	Insoluble fiber (g/ 100 g)	Total fiber (g/ 100 g)	Ash (g/ 100 g)
Whole wheat flour 10%	14.70	63.37	1.65	3.93	5.58	0.96
Whole wheat flour 20%	15.39	59.75	1.73	6.87	8.60	1.35
Whole wheat flour 30%	16.07	56.12	1.80	9.80	11.60	1.70

6.3.2.7 Statistical analysis

The effect of B&M content and addition time during kneading were assessed using a mixed effects model, as described by Montgomery, (2017). Specifically, an analysis of variance (ANOVA) was applied to each rheological and bread parameter to establish the significance of B&M content (fixed effect), addition time during kneading (random effect) and any interactions. In cases of statistically significant results ($p < 0.05$) a Tukey HSD post hoc test was performed. The three replicates were considered as a blocking factor.

6.3.3 *Results and discussion*

6.3.3.1 Rheological properties of dough

Table 23 recapitulates the results of the rheological tests and Table 24a summarizes the results of the statistical analyses.

Table 23: Results of alveographic tests performed according to three levels of bran and middlings content (10%, 20% and 30%) and five addition times during kneading: 0 (control), 2, 3.5, 5, and 6.5 min. Results are expressed as the mean of three replicates ± SD. Different letters represent statistically significant differences (p < 0.05). (Source: Cappelli et al., 2019a).

Sample	P (dough tenacity)	L (dough extensibility)	G (index of swelling)	W (deformation energy)	P/L
1) Whole wheat flour 10% addition time 0 (control)	65.13 ± 2.57^{b}	24.53 ± 0.92^{c}	11.20 ± 0.33^{a}	64.13 ± 3.41^{a}	2.80 ± 0.26^{b}
2) Whole wheat flour 10% addition time 2 min.	58.20 ± 1.22^{a}	29.60 ± 1.22^{a}	12.12 ± 0.24^{a}	62.33 ± 2.34^{a}	1.98 ± 0.11^{a}
3) Whole wheat flour 10% addition time 3.5 min.	54.33 ± 1.97^{a}	28.60 ± 5.60^{ab}	11.87 ± 1.15^{a}	57.13 ± 4.94^{a}	1.96 ± 0.36^{a}
4) Whole wheat flour 10% addition time 5 min.	62.93 ± 4.12^{b}	27.60 ± 4.42^{ab}	11.67 ± 0.92^{a}	65.47 ± 1.90^{a}	2.36 ± 0.49^{ab}
5) Whole wheat flour 10% addition time 6.5 min.	61.07 ± 1.47^{b}	26.00 ± 1.31^{b}	11.34 ± 0.31^{a}	61.93 ± 2.32^{a}	2.38 ± 0.17^{b}
6) Whole wheat flour 20% addition time 0 (control)	80.93 ± 5.02^{c}	18.27 ± 1.22^{d}	9.50 ± 0.31^{b}	63.33 ± 2.44^{a}	4.46 ± 0.53^{c}
7) Whole wheat flour 20% addition time 2 min.	78.20 ± 3.29^{c}	18.60 ± 2.82^{d}	9.56 ± 0.72^{b}	61.47 ± 5.50^{a}	4.34 ± 0.80^{c}
8) Whole wheat flour 20% addition time 3.5 min.	77.60 ± 3.56^{c}	17.93 ± 0.50^{d}	9.42 ± 0.13^{b}	60.27 ± 4.10^{a}	4.34 ± 0.15^{c}
9) Whole wheat flour 20% addition time 5 min.	79.67 ± 5.28^{c}	18.40 ± 0.40^{d}	9.54 ± 0.10^{b}	62.87 ± 4.30^{a}	4.33 ± 0.27^{c}
10) Whole wheat flour 20% addition time 6.5 min.	82.60 ± 3.41^{c}	19.00 ± 2.09^{d}	9.62 ± 0.41^{b}	66.00 ± 2.69^{a}	4.62 ± 0.25^{c}
11) Whole wheat flour 30% addition time 0 (control)	104.00 ± 4.65^{d}	15.33 ± 0.90^{d}	8.71 ± 0.25^{c}	69.13 ± 6.99^{b}	6.81 ± 0.42^{d}
12) Whole wheat flour 30% addition time 2 min.	109.93 ± 9.63^{d}	15.87 ± 0.42^{e}	8.86 ± 0.11^{c}	75.53 ± 8.60^{b}	6.94 ± 0.45^{de}
13) Whole wheat flour 30% addition time 3.5 min	111.87 ± 6.47^{de}	16.40 ± 1.78^{e}	8.98 ± 0.47^{c}	77.47 ± 5.31^{b}	6.92 ± 1.10^{de}
14) Whole wheat flour 30% addition time 5 min.	122.27 ± 5.42^{e}	16.00 ± 0.87^{e}	8.89 ± 0.24^{c}	83.87 ± 6.85^{b}	7.65 ± 0.48^{de}
15) whole wheat flour 30% addition time 6.5 min.	116.27 ± 7.48^{de}	15.27 ± 0.90^{e}	8.68 ± 0.25^{c}	77.73 ± 7.74^{b}	7.66 ± 0.36^{e}

Table 24: a) Alveograph parameters expressed as p-values. b) Bread characterization expressed as p-values. (–) indicates no statistical significance. (Source: Cappelli et al., 2019a).

Factor	P (Tenacity)	L	G	W	P/L
a) Chopin alveograph test					
Bran and middlings content	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Addition times during kneading	p 0.021	p 0.049	–	–	p 0.013
Bran and middlings content – addition times interaction	p 0.004	–	–	–	p 0.014

b) Breadmaking				
factor	Bread specific volume (L/kg)	Crumb density (g/ml)	Crumb moisture (%)	Crust moisture (%)
Bran and middlings content	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Addition times during kneading	p 0.017	-	–	–
Bran and middlings content – addition times interaction	–	–	p 0.001	–

6.3.3.2 Farinograph results

The results of farinograph tests show that WA increases as the percentage of B&M increases. Mean WA (percentage) was 55.50 ± 0.32, 59.10 ± 0.28 and 63.00 ± 0.25 for 10%, 20% and 30% WWF respectively. This is mainly due to the increasing B&M concentration, which increases total fiber content (Table 22) and WA capacity (Garofalo et al., 2011). Regarding DDT, 2.25 ± 0.14, 2.5 ± 0.10, and 3 ± 0.10 min were obtained respectively for WWF at 10%, 20% and 30%. This trend confirms that work of other authors (Cappelli et al., 2019a). Dough stability decreased as the percentage of B&M increased (5.85 ± 0.38, 4.5 ± 0.20, and 4 ± 0.30 min for WWF at 10%, 20% and 30% respectively). These findings are in accordance with those of other authors (Ahmed et al., 2013) and are related to the competition for disposal water and the incomplete hydration of starch and gluten caused by the increased fiber content (Table 22). Finally, TMD increased as the percentage of B&M increased, confirming the weakening of doughs found by other authors (Ahmed et al., 2013). This is due to an increase in total fiber content (Table 22).

6.3.3.3 Alveograph results

- Dough tenacity (P).

Regarding P, the ANOVA found significant individual effects of B&M content and addition time during kneading, and the interaction between them (Table 24a). In fact, the data reported in Table 23 shows that the effect of B&M content varied considerably as a function of addition time, notably for WWF at 10% and 20%. Gomez et al. (2011) and Cappelli et al. (2018) report that P increases with B&M content, which is related to an increased fiber concentration (Table 22) and which is linked, in turn, to a reduction in L values and an increase in dough viscosity and P (Garofalo et al., 2011).

Concerning 10% WWF, addition at 2 and 3.5 min resulted in a significant reduction in P compared to the control (Table 23). This amelioration following the delayed addition of B&M might be related to two phenomena. First, knead the dough for 2 or 3.5 min without interference (i.e. the addition of B&M) may improve starch hydration and the development of the gluten network, which gains in strength and resistance (Larsen, 1964) avoiding the water stealing related to water holding capacity of bran and middlings (Courtin and Delcour, 2002; Rosell et al., 2010) which are added later. Secondly, the addition of B&M at 2 or 3.5 min means that the mixture is kneaded for another 6 or 4.5 min, during which time the gluten network may manage the fracture of the structure (Gan et al., 1992) and steric hindrance of B&M (Hemdane et al., 2017). This hypothesis is supported by the literature. In particular, Zanoletti et al. (2017) argue that the energy provided during these 6/4.5 min is exploited for gluten rearrangement, which reduces the side effects of B&M addition. For the others addition times, no ameliorative effects were highlighted. This might be due to the short remaining kneading time after B&M addition, which does not allow a correct gluten rearrangement.

- Dough extensibility (L).

The results of the ANOVA for L show significant individual effects of B&M content and addition time, but not their interaction. As reported by Cappelli et al. (2018), L is significantly related to starch, gluten, and fiber content in ancient wheat flours. In the present study, L decreased as the percentage of B&M increased (Table 23). This decrease is linked to an increase in arabinoxylans and fiber concentration (Table 22), which reduces extensibility and increases viscosity and tenacity (Garofalo et al., 2011). Furthermore, increasing B&M concentrations are consistent with an increase in total protein content (Table 22) but reduced gluten concentrations (Pavlovich-Abril et al., 2016; Cappelli et al., 2018).

The results for 10% WWF highlight a clear increase in L with B&M addition at 2 and 3.5 min (Table 23). The delay has been found to maximize the hydration of starch and gluten (Larsen, 1964), while the addition of dough interferents significantly reduces gluten polymerization (Zhang et al., 2018), causing the fracture of the gluten structure (Gan et al., 1992) and significantly reducing L. The results reported by Noort et al. (2010) and Gan et al. (1992) support our findings; however, the physical interaction between fiber and the gluten network is the main driver of the negative effects of B&M addition on L. Addition at 2 min improves starch and gluten hydration and the additional 6 min of kneading may have the same effects on the gluten network observed for P.

For 20% and 30% WWF, the beneficial effects of the delayed addition of B&M are not statistically significant. This could be attributed to the dominant effect of the percentage of B&M which, as reported by Zhang et al. (2018) and Garofalo et al. (2011), determines depolymerization of the gluten network and fracture of the structure. In this case, gluten can only partially manage the negative effects of B&M addition through rearrangement, with an inevitable fall in L.

- Index of swelling (G).

With regard to G, the results of the ANOVA highlight a significant individual effect of B&M content, while this is not the case for addition time or their interaction. As earlier work (Cappelli et al., 2018) reports, G is related to starch, gluten, and insoluble fiber content in ancient wheat flours. Table 23 shows that G decreased as the percentage of B&M increased. This is linked to a reduction in L due to an increased insoluble fiber concentration (Table 22), which is linked to a reduction in G and an increase in viscosity and tenacity (Cappelli et al., 2018; Garofalo et al., 2011). Furthermore, this increase is correlated with an increase in total protein content (Table 22), but a decrease in gluten (Pavlovich-Abril et al., 2016; Cappelli et al., 2018).

- Deformation energy (W).

Concerning W, the ANOVA found a significant individual effect of B&M content, but not addition time or the interaction. The addition of B&M increases dough viscosity (Le Bleis et al., 2015) and tenacity (Gomez et al., 2011), which is linked with decreased extensibility (Garofalo et al., 2011). Furthermore, increasing B&M is consistent with increased total protein content (Table 22) but reduced gluten (Pavlovich-Abril et al., 2016; Cappelli et al., 2018). Table 23 shows an increase in W as B&M increases from 10% to 30%; this result is in accordance with earlier work (Cappelli et al., 2018; Gomez et al., 2011). This increase in W may be due to a change in the area under the curve of the graph. In fact, the very marked increase in P and the smaller reduction in L increase the area under the curve (i.e. W values) in 30% WWF and the latter percentage had no ameliorative effects on bread characteristics.

- Curve configuration ratio (P/L).

Regarding P/L, the ANOVA found significant individual effects of B&M content and addition time, and their interaction (Table 24a). The P/L ratio is an alveograph parameter that is closely correlated to the technological success of leavened products. An increase in the P/L ratio found in WWF and type 2 flours is one of the major reasons for inferior bread characteristics (Parenti et al., 2019) and is particularly marked in ancient wheat flours. It is therefore important to highlight the significant interaction between the two factors. Despite is hard to highlight a clear trend, Table 23 shows that the effect of B&M content varies considerably as a function of the five addition times. In particular, the P/L ratio decreases at 2 and 3.5 min for 10% WWF, but increases at 5 and 6.5 min for 30% WWF.

Furthermore, the ANOVA identifies a significant individual effect of B&M content. Table 23 shows that an increase is consistent with an increase in P/L. This increase can be explained by variation in both P and L. In particular, higher B&M content significantly increases P, while the decrease in L is modest, as highlighted in earlier work (Cappelli et al., 2018; Garofalo et al., 2011). P/L falls significantly for 10% WWF with the addition of B&M at 2 and 3.5 min. The reason for this is the same as observations regarding P and L. The first few minutes of kneading without interferents allow starch and gluten hydration, with positive effects on L (Larsen, 1964), while the remaining kneading time allows the gluten network to manage the negative effects of B&M addition, reducing P. However, this delay cannot be too long, as the gluten network needs time and energy to manage the negative effects of bran and middlings addition through gluten rearrangement (Zanoletti et al., 2017). For 20% WWF, the ameliorating effect of delayed B&M addition is less pronounced but remain observable at 2, 3.5, and 5 min where P/L is lower than the control (0 min).

Regarding 30% WWF, no reduction was found in P/L for any of the tested addition times. This could be attributed to the insurmountable negative effects of this high percentage of B&M, leading to a huge increase in P and decrease in L (Cappelli et al., 2018; Garofalo et al., 2011). In this case, gluten is unable to manage the negative effects of the high amount of B&M, with an inevitable hike in P/L. P/L values at 2 and 3.5 min are very similar to the control, while they are significantly higher for addition at 5 and 6.5 min. These results suggest that addition at 2 or 3.5 min is most promising, and extending the total kneading time could slightly lower P/L.

6.3.3.4 Bread characteristics

Table 25 summarizes results regarding bread specific volume, crumb density, and crumb and crust moisture. Table 24b shows the results of the statistical analyses.

Table 25: Bread characterization as a function of bran and middlings content (10%, 20% and 30%) and five addition times during kneading: 0 (control), 2, 3.5, 5, and 6.5 min. Results are expressed as the mean of three replicates ± SD. Different letters represent statistically significant differences (p < 0.05). (Source: Cappelli et al., 2019a).

Sample	Specific volume (L/kg)	Crumb density (g/ml)	Crumb moisture (%)	Crust moisture (%)
1) Whole wheat flour 10% addition time 0 (control)	2.96 ± 0.05^{b}	0.36 ± 0.04^{a}	42.36 ± 1.05^{a}	24.18 ± 0.76^{a}
2) Whole wheat flour 10% addition time 2 min.	3.13 ± 0.09^{a}	0.32 ± 0.09^{a}	43.54 ± 0.79^{a}	27.17 ± 0.73^{a}
3) Whole wheat flour 10% addition time 3.5 min.	3.02 ± 0.11^{ab}	0.31 ± 0.08^{a}	42.70 ± 0.42^{a}	25.34 ± 0.61^{a}
4) Whole wheat flour 10% addition time 5 min.	2.90 ± 0.10^{b}	0.33 ± 0.07^{a}	43.74 ± 1.56^{a}	25.30 ± 0.94^{a}
5) Whole wheat flour 10% addition time 6.5 min.	2.94 ± 0.07^{b}	0.36 ± 0.07^{a}	44.30 ± 0.16^{a}	26.07 ± 1.00^{a}
6) Whole wheat flour 20% addition time 0 (control)	2.33 ± 0.08^{d}	0.39 ± 0.05^{b}	45.10 ± 0.25^{b}	28.72 ± 1.52^{b}
7) Whole wheat flour 20% addition time 2 min.	2.46 ± 0.09^{cd}	0.39 ± 0.06^{b}	44.46 ± 0.68^{b}	27.94 ± 1.26^{b}
8) Whole wheat flour 20% addition time 3.5 min.	2.43 ± 0.19^{cd}	0.41 ± 0.06^{b}	44.26 ± 0.81^{b}	27.68 ± 1.62^{b}
9) Whole wheat flour 20% addition time 5 min.	2.51 ± 0.12^{cd}	0.47 ± 0.17^{b}	44.57 ± 0.28^{b}	28.93 ± 1.19^{b}
10) Whole wheat flour 20% addition time 6.5 min.	2.43 ± 0.10^{c}	0.51 ± 0.20^{b}	44.32 ± 0.57^{b}	27.79 ± 1.17^{b}
11) Whole wheat flour 30% addition time 0 (control)	1.95 ± 0.07^{cd}	0.48 ± 0.13^{c}	45.47 ± 0.18^{c}	28.62 ± 2.27^{c}
12) Whole wheat flour 30% addition time 2 min.	2.06 ± 0.05^{e}	0.45 ± 0.10^{c}	46.03 ± 0.81^{c}	29.51 ± 1.68^{c}
13) Whole wheat flour 30% addition time 3.5 min	2.01 ± 0.08^{e}	0.52 ± 0.15^{c}	45.66 ± 0.88^{c}	29.23 ± 1.78^{c}
14) Whole wheat flour 30% addition time 5 min.	2.00 ± 0.09^{e}	0.57 ± 0.03^{c}	46.16 ± 0.41^{c}	29.64 ± 1.10^{c}
15) whole wheat flour 30% addition time 6.5 min.	1.97 ± 0.08^{e}	0.47 ± 0.07^{c}	45.76 ± 0.92^{c}	30.70 ± 1.39^{c}

- Bread specific volume

The results of the ANOVA for bread specific volume found significant individual effects of B&M content and addition time, but not the interaction (Table 24b). As highlighted by Gomez et al. (2011), Gan et al. (1989), and Packkia-Doss et al. (2019) bread volume decreases as the percentage of B&M increases (Table 25). This significant reduction is related to the effects of non-endosperm components of the caryopsis. In particular, as reported by Gan et al. (1992), it is provoked by the disruption of the starch–gluten matrix caused by epicarp hairs (along with other non-endosperm components). This damage reduces specific volume and increases WWB crumb density (Gan et al., 1992). This is consistent with the results reported in Table 25.

Regarding addition time, Table 25 shows that for 10% WWF bread specific volume was highest at 2 min. This was also the case for 20% WWF, although the results are more nuanced. Although bread specific volume was higher than the control at 2 min, it was highest at 5 min. For 30% WWF the delayed addition of B&M significantly increases the bread specific volume (best performance at 2min) It is important to highlight that these results reflect those obtained for the alveograph parameters P, L and P/L, and underlines the accuracy of the rheological assessment and the influence of these parameters on bread characteristics. These results for bread specific volume can be attributed to the phenomena reported above. The hypothesis that the negative effects of B&M addition are related primarily to the physical interaction between non-endosperm components and the gluten network is also confirmed by other authors (Noort et al., 2010; Gan et al., 1992). Together, these findings support the ameliorative effect of the delayed addition of B&M at 2 min.

- Crumb density

Concerning crumb density, the ANOVA shows significant individual effects of B&M, while this is not the case for addition time or their interaction (Table 24b). Table 25 reports that at 20% and 30% B&M, crumb density increases significantly. This finding is in accordance with the results presented in Zanoletti et al. (2017), which highlight that crumb density increases as the percentage of B&M increases. As reported by Courtin and Delcour (2002), the addition of large amounts of B&M increases both crumb density and hardness. This is mainly due to the effects of arabinoxylans, inulin, and β-glucans on dough rheology and bread characteristics (Courtin and Delcour, 2002; Cappelli et al., 2019a).

- Crumb and crust moisture

The results of the ANOVA for crumb moisture found a significant individual effect of B&M content. This was not found for addition time, although a significant interaction was found (Table 24b). This interaction underlines the importance of the proposed strategy. In this case, the effect of B&M content varied considerably as a function of the addition time, particularly for 10% and 30% WWF (Table 25). As highlighted by Curti et al. (2013) and Zanoletti et al. (2017), a progressive increase in B&M concentration leads to a significant increase in crumb moisture. This is consistent with the results reported in Table 25. Increased crumb moisture might be attributable to the amount of water added, the progressive increase of water holding capacity (Zanoletti et al., 2017) and the weaker solid–liquid interaction, due to the increase in fiber (Table 22).

Regarding crust moisture, the results of the ANOVA show a significant individual effect of B&M content, while this was not the case for addition time or the interaction. Although Curti et al. (2013) do not find a significant variation in crust moisture, the results reported in Table 25 show an increase in crust moisture as the percentage of B&M increases. The increase in crust moisture with higher amounts of B&M (i.e. 20% and 30%) could be related to the increased water holding capacity of B&M with respect to refined white flour.

6.3.4 Conclusions

The results presented in this research show the effectiveness of the proposed strategy in improving whole wheat dough kneading. The delayed addition of B&M during kneading is able to influence both dough rheology and bread quality. Regarding rheology, the proposed technique is able to reduce dough tenacity (P), increase dough extensibility (L) and consequently reduce P/L, which is crucial for the technological success of leavened products. The ameliorative effects on P/L are very interesting, in as much as the increase in the tenacity/extensibility ratio is one of the most problematic defects of whole wheat bread.

Furthermore, the positive effects of the proposed strategy are not limited to dough rheology – beneficial effects are also found for whole wheat bread characteristics, notably increased bread specific volume.

For 10% whole wheat flour, performance is best for addition at 2 min (i.e. 25% of total kneading time), and clearly superior than the control. Although, addition at 3.5 min (i.e. 43.75% of total kneading time) also performed well, the decrease in W suggests that addition at 2 min is more suitable. The findings support the hypothesis of gluten rearrangement. Addition at 2 min means that the dough is kneaded for a further 6 min (compared to 4.5 min with addition at 3.5 min) with beneficial effects on the gluten network.

Similarly, for 20% whole wheat flour, addition at 2 min appears to be the best strategy to enhance whole wheat dough rheology and, especially, to improve whole wheat bread characteristics. Finally, for 30% whole wheat flour, no marked ameliorative effects were found, except for bread specific volume. Nevertheless, even in this case, addition at 2 min resulted in highest bread specific volume and best crumb density.

In conclusion, the proposed strategy is able to ameliorate whole wheat dough rheology and whole wheat bread characteristics. Other advantages include the ease of application and the lack of additional monetary expenditure. Moreover, it could make flour storage easier and help millers to simplify the management of whole wheat flours orders. Although the phenomenon should be investigated in greater depth, our work could guide the development of specific kneading machines for whole wheat flour, with the aim of enhancing whole wheat dough rheology and whole wheat bread characteristics through the correct management of the kneading phase.

7 Ex-post: innovations and improvements linked to the sustainability of flours, pasta, and bakery production

7.1 Use of LCA analysis to assess the environmental sustainability of pasta production chains: an integrated approach for comparing local and global chains

7.1.1 Aim of the study

The environmental impact of food productions, in particular of wheat pasta, can be significant, and this has pushed major pasta industries to start evaluating the environmental footprint of their productions by means of Life Cycle Assessment (LCA) and, in some cases, even by Environmental Product Declaration (EPD), according to the standards of the International Organization for Standardization (ISO standards) (ISO 14040, 2006), making information widely available. The reasons supporting this choice are mainly due to the increasing attention of final consumers on the possible impacts of industrial production on the environment, which determined a growing public pressure on this thematic. Furthermore, the renewed interest of consumers in ancient grains has promoted an increase in their cultivation and use, expanding the number of products offered by the baking industry (Cappelli et al., 2018; Cappelli et al., 2019a). This has also contributed to the safeguarding of biodiversity and to the development of a local micro-economy, which allows local producers to increase their profits by differentiating their products (Cappelli et al., 2018; Cappelli et al., 2019a).

Bevilacqua et al. (2007), identify these life cycle phases in the whole production process of pasta: durum wheat cultivation; milling of durum wheat to obtain semolina; pasta production and packaging; transportation and distribution of final products; domestic consumption, waste and pallet disposal (Recchia et al., 2019). These phases are also mentioned and quantified in the product category rules and reports of the International EPD® System for industrial pasta producers. Wheat cultivation is the most variable and, at the same time, fundamental stage. Tillage and all other operations involving soil treatment have the purpose of creating favorable conditions for seed germination and growth, exploiting different techniques. Traditional practices used in Italy chop residuals of the previous crop, use a moldboard plough to dig the ground at a depth of 0.30 to 0.35 m, and harrow it with one or more passages of a disc harrow. All these operations are carried out as soon as possible after harvesting the previous crop (typically on July, in central Italy). Sowing of durum wheat is done in mid-autumn with the use of a universal seed drill, and, more rarely, employing pneumatic seeders. The spacing between rows varies from 0.14 to 0.18 m while seed depth varies between 20 and 50 mm. The quantity of distributed seed is approximately 180 to 200 kg per hectare. In durum wheat cultivation performed on a large industrial scale, a minimum tillage is often used with herbicide application before seeding to control weeds. In this case, sowing is done with a direct or combined seed drill, which typically releases up to 220 kg of seed per hectare at a depth of about 50 mm and a 0.2 to 0.3 m row spacing, even if much lower quantities are used in some countries, such as in southern Australia. Fertilizers are applied at variable rates, depending on the soil characteristics.

In this work, the environmental sustainability of two different pasta production chains was evaluated: The first concerns the production of "high-quality pasta" which is accomplished by following traditional production procedures on a Tuscan farm that use only ancient wheat varieties (referred here as "local or regional scenario") (Recchia et al., 2019); in the second, a "conventional pasta" is produced using national and international grains and following industrial processes (referred here as "global or industrial scenario") (Recchia et al., 2019). Results of this analysis are presented and discussed here, comparing the two chains in terms of their environmental impacts throughout the whole production process, and drawing some conclusions. Moreover, an integrated methodology based on an Environmental Impacts ANalysis (EIAN) and the LCA has been developed, investigating five environmental compartments (i.e. soil, water, air, resources, climate change) and a total number of ten expected environmental pressures. This developed methodology assures a comprehensive environmental evaluation of the products: Using EIAN approach, the site-specific impacts can be assessed through qualitative and quantitative indicators, to integrate the results usually obtained by the LCA or the EPD implementation (Recchia et al., 2019).

7.1.2 Materials and methods

7.1.2.1 Description of the Methodology for the Environmental Assessment

This work assesses the environmental pressures associated with the production of high-quality and conventional pasta. These two production systems imply different approaches in terms of agricultural management, logistics, industrial plant optimization, etc. To achieve this goal, an integrated methodology based on both site-specific and global evaluations has been developed. A number of both quantitative and (few) qualitative indicators have been considered to investigate the different characteristics of the two production systems, as well as their environmental impacts. Several European projects working on biofuels production adopt a similar methodology integrating local impacts evaluation with the LCA, not only for the agricultural phase but also for the logistic and industrial phases (Bevilacqua et al., 2007).

This approach can assure a comprehensive environmental assessment of the products. The present study considered 5 different environmental compartments (i.e., soil, water, air, resources, climate change) and a total number of 10 expected environmental pressures, as illustrated in Fig. 17. For each pressure, specific indicators were set with the aim of evaluating both local and global consequences of production processes. Table 26 illustrates the environmental indicators considered for each production phase as regards the analyzed environmental pressures. Afterwards, indicators are evaluated for each of the proposed scenarios to compare them in terms of their performance, with respect to the environmental pressures. A comprehensive performance is defined by assigning a value of 2 (worst performance), 1 (equivalent performance) or 0 (better performance) for each indicator, and, then, by summing all values related to each pressure, and obtaining a total score for it. The higher each score is, the lower the corresponding performance. Lower performances are highlighted in red, while higher performances are colored in green; equivalent scores correspond to equivalent performances and are colored in yellow. As a consequence, it is possible to compare the two scenarios considered in this study.

A - Soil	**B - Water**	**C - Air**	**D - Resources**
- Land use (A1)	- Pollution (B4)	- Pollution (C6)	- Biotic: agrobiodiversity (D8)
- Pollution (A2)	- Abstraction or Diversion (B5)	- Noise (C7)	- Abiotic: non renewable (D9)
- Degradation (A3)		**E - Climate change** (E10)	

Fig 17: Environmental compartments and pressures under investigation. (Source: Recchia et al., 2019).

Table 26: Environmental Impacts ANalysis (EIAN) and Life Cycle Assessment (LCA) indicators considered for the environmental assessment. (Source: Recchia et al., 2019).

Investigated Aspect	Environmental Pressure	Local Indicator (EIAN Approach)	Global Indicator (LCA Methodology)
1. Agricultural phase			
Crop choice	A1	- average yield	-
	A2, B4	- agrochemicals typology and quantity	-
	B5	- WUE of cultivar	- CMR
	D8	- adoption of autochthon cultivars	-
Soil management	A3	- mechanization level	-
	D9	- fuel volume	- CER, CMR
	E10	-	- CO_2eq emissions
Fertilisers use	B4	- fertilizers typology and quantity	- Eutrophication
	D9	- fossil fertilizers quantity	- CER, CMR
	E10	-	- CO_2eq emissions
Pesticides use	A2, B4, D8	- pesticides risk index	- Eutrophication
	B5	- dilution water volume	- CMR
	D9	- pesticides typology and quantity	- CER, CMR
	E10	-	- CO_2eq emissions
2. Pasta production phase			
Industrial plant management	B5	- water volume	- CMR
	C7	- technology level of the equipment	-
	D9	- electricity consumption	- CER, CMR
	E10	-	- CO_2eq emissions
3. Transport phase			
Transports of grains, semolina and pasta	C6	- logistic optimization level	-
	C7	- transport means typology	-
	D9	- fuel volume	- CER, CMR
	E10	-	- CO_2eq emissions
4. Cooking phase			
Domestic use	D9	- energy consumption	- CER, CMR
	B5	- cooking water volume	- CMR
	E10	-	- CO_2eq emissions

7.1.2.2 EIAN Approach and Indicators

The aim of the EIAN is the evaluation of comprehensive and potential impacts on a site-specific base, hypothesizing the interaction between the production system and the environment at the local scale. To reach this objective, several quantitative and/or qualitative indicators have been set. This approach takes inspiration from the Environmental Impact Assessment methodology as described in Italian and European legislation, but it is not at all the same administrative and technical procedure. The proposed approach allows to evaluate several pieces of information about the production chains which can potentially compromise the environment at a site-specific level, and afterwards integrates them with the potential global impacts assessed by the LCA. For the agricultural phase, the EIAN approach was implemented following the indications reported in Rettenmaier et al. (2014).

As far as the crop choice is concerned, it is important to consider the plant and the environment as an integrated system, through particular site-specific indicators: In fact, crops highly suited to specific geographical areas are assumed to be more resistant to pests and climate variability. Moreover, crops, which are typically cultivated in a specific area, assure lower risks of biodiversity losses if compared to allochthonous crops or cultivars, introduced from other environments. On the other hand, the use of ancient cultivars could imply lower yields and lower efficiencies in water use, reducing the available water for agricultural uses in a specific geographical area, especially if the agricultural management is not optimized.

Soil management is also important for assessing environmental sustainability of the process: Crops characterized by a well-developed root system and the burying of agricultural residues are assumed to decrease the risk of erosion for a similar crop soil coverage. Erosion causes a loss of organic soil substances and leads to a loss of habitats, reducing water filtering and buffering functions with potentially negative effects on biodiversity. Moreover, lower levels of mechanization, if implemented, could assure a lower risk of soil fertility, compaction, and biodiversity losses, higher carbon matter storage, lower atmospheric emissions, and reduced energy requirements, etc. In any case, tillage operations, which assure an optimal soil structure and an adequate diffusion of liquids, can contribute to a more efficient use of water field resources and soil fertility preservation.

The type and the quantity of fertilizers can not only directly affect water quality, gaseous emissions, and biodiversity but also the soil's physical and chemical characteristics. At the same time, the spreading of the fertilization compounds implies direct emissions in agricultural land (e.g., atmospheric emissions, noise, soil compaction, etc.). Similar considerations can be made for pesticides, whose risks were evaluated according to the simplified approach elaborated by the Regional Agency for the Environment Protection in Tuscany, Italy (referred here as "ARPAT model") (ARPAT, 2015), hypothesizing the quality level of the waters and ecosystems of the considered geographical area. The ARPAT model sets three different indicators: the overall impact, the water impact, and the ecosystem impact. The model accuracy is comparable to that one of more complex models, which require significant effort and skill in analyzing all the active chemicals used and their effects on the environment (soil, water and organisms) (ARPAT, 2015). In Reference to ARPAT (2015) a useful list of the most common active chemicals is reported, indicating 3 impact indicators for each one; the overall expected risks for weeding and phytosanitary treatments can be calculated by multiplying these indicators per the dose of the active compound.

As far as the logistics of the production system is concerned, a direct influence on atmospheric emissions, fossil fuel requirement, noise emissions during transport and biodiversity, too, is given by transport distances, typology of means of transport, optimization level, and the potential increase of induced traffic at a local level. Production processes and cooking phase are eventually responsible for raw materials and energy consumptions, and atmospheric emissions, too.

7.1.2.3 LCA Methodology and Indicators

Together with the evaluation of site-specific indicators (Table 26), a comparative LCA was carried out with the aim to evaluate global pressures caused by production processes, highlighting the main differences between high-quality and conventional pasta production processes. Attention was paid to single out which phases are more critical from an environmental point of view, and then possible solutions to reduce their impact are proposed. LCA was carried out according to the ISO 14040 standard (ISO 14040, 2006), by modeling the production chain through the Gemis® software version 4.95 (IINAS GmbH, Darmstadt, Germany) which consists of an open source analysis model and a database (Gemis, 2018). Moreover, the Biograce® GHG calculation tool version 4d (IFEU GmbH, Heidelberg, Germany) (Biograce, 2018), was also used for calculating N2O emissions due to the use of fertilizers. This methodology was applied to determine the following environmental pressures:

1. Effects on global warming, by quantifying CO_2 equivalent emissions (CO_2eq), accounting for carbon dioxide (CO_2), methane (CH4) and nitrous oxide (N2O) emissions, and using the Global Warming Potential GWP100 factors;
2. Primary energy consumption, using the Cumulated Energy Requirement (CER), which represents the fossil energy required for extracting, manufacturing and disposal of raw and auxiliary materials;
3. The Cumulated Material Requirement (CMR), by considering all the non-renewable raw materials used throughout the whole life cycle;

4. The Eutrophication index, by considering nutrients leaching in superficial and ground water.

LCA indicators used were limited to 4 to reduce the amount of inventory data necessary to perform the present analysis. Actually, the collection of additional data to take more indicators into account would require a significant effort in terms of time and resources, which were not available and could not be performed by all firms involved in the local production system, save by a simple estimation (Bevilacqua et al., 2007). The 4 indicators were selected considering some results available in the literature. In fact, food, drink, tobacco, and narcotics areas account for an estimated 20 to 30% of most environmental impacts, except for eutrophication, which reaches 59% (Foster et al., 2006). This result is well known, even if environmental impacts arising across the entire life cycle ("from farm to fork") were studied in detail only for a few basic or processed foods. Actually, in the field of pasta production, specific studies have been carried out only in the last decades. This can be explained since plant-based products are those with lower impact levels. For example, cereal, bread, flour, and related products account for a little more than 1% of the EU's global warming potential (GWP) and of photochemical ozone creation potential, while the eutrophication potential is about 9% (Foster et al., 2006).

7.1.2.4 System Boundaries and Functional Unit

Both pasta production chains analyzed here consist of the following stages: durum wheat cultivation; milling of durum wheat to obtain semolina; pasta production and packaging; transportation, and wastes management. In the local scenario, it is necessary to account for an additional transport phase required to bring the product from the processing plant back to the farm where the pasta is marketed. Effects of the distribution phase from the producing industry to the final user were only estimated considering the different radius of the selling areas starting from the production plants for the two proposed chains. Domestic cooking consumptions were experimentally investigated for the high-quality pasta, while a simple estimation was considered for the conventional pasta.

In Fig. 18 the main inputs (product) and outputs (residues) considered for environmental evaluations carried out in the present work are reported. However, a number of inputs and outputs have been excluded: materials needed for construction and maintenance of machines and equipment; construction materials for farm buildings and machines; transport associated with fertilizers and other agrochemicals delivered to the farm; depuration treatments of production plant wastewater; energy and resources consumptions due to administrative activities; ink consumption and printing for the packaging of the product. Moreover, according to the Danish Environmental Protection Agency (2017), from 11% to 33% of cooked pasta is wasted on average, with even higher amounts occurring in European restaurants and schools. As a consequence, due to its high variability, in this work, the environmental impacts of food waste were not considered.

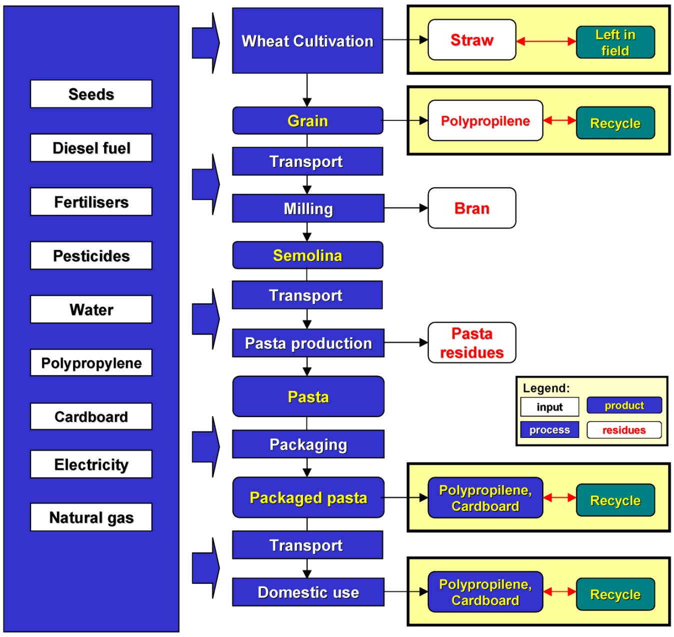

Fig 18: System boundaries of the pasta production. (Source: Recchia et al., 2019).

As a functional unit (FU), 1 kg of dried pasta was considered. In particular, for the traditional pasta the whole system was modeled considering an annual production of 1500 kg of dried pasta, which represents the marketable product whose experimental data for the production of the traditional pasta are referred. This quantity is representative of the very small Italian firms which produce ancient grains pasta. In fact, the Italian statistical data (Agristat, 2018), show that more than 50% of the Italian farms have agriculturally used land which measures less than 2 ha and, if the land is suitable for wheat cultivation, they choose ancient grains crops to obtain higher economic revenues. Instead, for the conventional pasta, an average production capacity of 75 t/day (BREF, 2006) was assumed.

7.1.2.5 Inventory Data Collection

For what concerns the local scenario for high-quality pasta, the inventory data were collected in the three firms constituting the production chain, all located in Central Italy: Montepaldi farm (Firenze, Tuscany, Italy) for wheat cultivation; Molino Silvestri (Torgiano, Umbria, Italy) for wheat milling; Pastificio Artigianale Fabbri (Strada in Chianti, Tuscany, Italy) for pasta production. The pasta is commercialized with the Montepaldi brand. Data referred to the annual production of Montepaldi pasta. Agricultural data were collected through a questionnaire and represent the average mean of the years from 2012 to 2015, while, for the other phases, data were collected through direct measurements during the 2012 campaign.

No significant differences were observed in terms of plant efficiency and input/output quantities during the observation period, so no criticisms emerged in the modeling process. However, since pasta is one of the most popular and widespread foods, its market demand is predominantly satisfied by conventional pasta, with a growing interest by consumers for conventional organic pasta (Babula & Rich, 2001). In fact, pasta made with ancient grains and other special high-quality pasta typologies can only satisfy local and regional demands, in terms of attention to environmental sustainability and due to the lower production capability of local producers. For this reason, an industrial scenario supplying conventional pasta on a global scale was investigated, too. Finally, for both local and global scenarios the cooking phase was included: For the high-quality pasta, experimental data were used, while for the conventional pasta estimated data were considered.

7.1.3 Results

7.1.3.1 Description of Data Inventory

Table 27 shows the data for both the high-quality (local) and conventional (global) pasta production scenarios. Concerning the local scenario, based on cultivation of ancient wheat cultivars and small-sized farms for the semolina and pasta production, an average yield of 2450 kg/ha was calculated, accounting for a +/-36% of variability in the period from 2012 to 2015 due to different climatic condition. The wheat cultivar used in the farm under observation for the local scenario is "Senatore Cappelli", one of the most used *Triticum durum* cultivars in Italy, especially in Tuscany, even if other cultivars are becoming popular (e.g., "Timilia" and "*Triticum turgidum*", typical of Sicily). These old cultivars have a lower average yield with respect to modern ones (Cappelli et al., 2018; Cappelli et al., 2019a), even if the quantity of sowing seed is similar, ranging from 200 to 220 kg/ha.

The level of mechanization is normally higher for more intensive conventional wheat cultivation, due to the higher number, power, and weight of agricultural machines operating in field, which is characterized by a larger surface and higher accessibility (e.g., lower slope). Considering the fertilization inputs, even if there is not much difference in the use of potassium between the two scenarios, the conventional wheat production requires almost two times the nitrogen normally applied in the old variety cultivation (an average amount of 220 kg/ha vs. 114 kg/ha), because of its higher production potentialities and the need to minimize the risk of low yield without caring about the risk of lodging.

Moreover, the quantity of fertilizers for the Montepaldi farm are reduced since in this farm only one nitrogen fertilization is usually carried out. Pesticides are used in similar quantities, too. Significant differences have been detected in comparing the pasta production phase. Water consumption turned out to be higher for the high-quality pasta chain, which needs a water volume of 0.415 kg/FU for the pasta production and the remaining quantity for the washing of equipment and machines. Contrariwise, for conventional pasta, the volume of water used for cleaning is significantly reduced because of larger quantity of pasta produced and the use of compressed air instead of water in the majority of the equipment. Therefore, in this case, the quantity of water needed for manufacturing pasta was similar, according to values reported in the literature, ranging between 22 and 30 kg per 1 kg of semolina (BREF, 2006).

Table 27: Inventory data for high-quality and conventional pasta production. (Source: Recchia et al., 2019).

Typology of the Pasta	High-Quality/Traditional	Commercial/Conventional
Typology of the chain	local/regional	global
Data source	Montepaldi pasta system	literature
Reference years	2012; 2013; 2014; 2015	-
Geographical location	Central Italy	-
Agricultural phase:		
(1) Crop cultivar	Senatore Cappelli; Timilia; Triticum turgidum	-
(2) Average yield [kg/ha]	2450	4000 (1)
(3) Seeds sowing [kg/FU]	0.151	0.074 (2)
(4) Agricultural operations		
- Operations typology	Plowing; harrowing; sowing; fertilizing; weeding and phytosanitary treatments; irrigation (optional); harvesting; straw shredding; transports	Sowing; fertilizing; weeding and phytosanitary treatments; irrigation (optional); harvesting; straw shredding; transports
- Diesel fuel consumption [MJ/FU]	2.718	2.023 (3)
(5) Fertilization		
- Fertilizers typology	Biammonic phosphate 18/46; Nitrogen 46%; P2O5 18%; Ammonium nitrate N 27%; Urea N 46%	Nitrogen 46%; P2O5 18%; Urea N 46%
- Total nitrogen volume [kg/FU]	0.065	0.074 (2)
- P_2O_5 quantity [kg/FU]	0.021	0.010 (2)
(6) Plant treatments:		
- Herbicides typology	Axial; Granstar Ultra 50	Tribenuron-methyl; pinoxaden
- Insecticides/fungicides typology	Novel Duo; Binal Pro; Amistar extra	Propiconazole; azoxystrobin;
- Total pesticides quantity [kg/FU]	0.0011	0.0007 (2)
- Water consumption [kg/FU]	0.757	0.297 (2)
(7) Residues production:		
- Residues management	- shredded in field	- shredded in field
- Residues quantity [kg/FU]	2.533	1.760 (1)
(8) Grain packaging		
- Polypropylene big-bag [kg/FU]	0.008	0.004 (4)
Pasta production phase:		
(11) Water consumption [kg/FU]	7.735	0.400 (5)
(12) Electricity consumption [MJ/FU]	1.027	0.830 (5)
(13) Pasta packaging		
- Polypropylene [kg/FU]	0.019	0.023 (4)
- Cardboard [kg/FU]	0.232	0.278 (4)
Transports distances:		
(9) from field to gate [km]	560	700 (4)
(10) from plant to consumers [km]	75	2000 (4)
Cooking phase:		
(14) Water requirement [kg/FU]	10	10 (4)
(15) Energy consumption [MJ/FU]	9.010	15.034 (4)

Considering the transport phase, the high-quality pasta chain was less optimized in term of choice of means of transport and pathways, even if the distances were reduced. Particularly, for the local scenario, a significant energy consumption was required to cover a distance of 19 km from the pasta production plant to the Montepaldi farm, with 12 movements needed for moving the produced pasta. Concerning the global system, the transport distances were assumed considering an average comprehensive amount of 2.700 km shared in agricultural trucks (4%), road transport (45%) and ship transport (51%), and an exporting rate of 25%, for example, in North America.

Energy requirements for the domestic cooking of the Montepaldi pasta were measured carrying out an experimental test because of the lack of bibliographic data for cooking of ancient grain pasta: 300 g of pasta were cooked in 3 l of water with 30 g of salt consuming 53 g of butane in 14 minutes. For the conventional pasta, the cooking phase was modeled considering an energy consumption of about 15 MJ/FU and a required amount of 10 kg/FU as reported in (Bevilacqua et al., 2007). The difference in the energy consumption between the two typologies of the pasta is probably due to the different cooking conditions (e.g., energetic source, pasta quantity, and type and shape of cooked pasta, etc.) and with respect to the cooking time. In fact, optimized cooking conditions are more easily assured for the traditional pasta due to its higher economic value which determines higher attention in the cooking phase to preserve the characteristics of the pasta and fulfill the consumers' expectations.

7.1.3.2 Results of the Environmental Assessment

Table 28 shows the results of the application of the integrated EIAN-LCA methodology presented above. In the agricultural phase, the high-quality pasta chain achieves a better performance due to a lower level of mechanization and a lower consumption of fuel, fertilizers, and pesticides. Similarly, the risk of pesticides diffusion in the ecosystems is lower, according to results obtained from the model proposed by ARPAT (Regional Agency for the Environment Protection in Tuscany, Italy) (ARPAT, 2015) (Table 29). However, the commercial pasta chain requires a lower volume of water since the cultivation of modern cultivars is characterized by a higher WUE and exploits more efficient sprayers which allows one to use lower water volumes. Similar considerations can be made for water and electricity requirements in the industrial phase due to the larger plant sizes, the optimized equipment, and economy of scale.

Table 28: Results of the integrated EIAN-LCA methodology. (Source: Recchia et al., 2019).

Production Chain	High-Quality/Traditional Pasta	Commercial Pasta
1. Agricultural phase		
- average yield [kg/ha]	2450	4000
- WUE of cultivar	Low	high
- adoption of autochthon cultivars	Yes	no
- mechanization level	medium	high
- fuel volume per ha	4 GJ/ha	6 GJ/ha
- agrochemicals [kg/ha]	149	252
- fertilisers [kg/ha]	147	250
- dilution water [kg/ha]	1113	880
- pesticides quantity per ha	1.9	2.2
- pesticides risk index	2.83 – 3.05 – 2.52	4.40 – 4.22 – 3.17
- CO_2eq emissions [g/FU]	1236	1217
- CER [MJ/FU]	7.2	6.6
- CMR [kg/FU]	0.176	0.117
- Eutrophication [mg/FU]	7.264	5.242
2. Pasta production phase		
- technology level of the equipment	low	high
- water volume [kg/FU]	7.735	0.400
- electricity amount [kg/FU]	1.027	0.830
- CO_2eq emissions [g/FU]	253	245
- CER [MJ/FU]	3.6	3.7
- CMR [kg/FU]	0.028	0.033
3. Transport phase		
- logistic optimization level	low	high
- transport means typology	agricultural truck + road transport	agricultural truck + road transport + ship transport
- fuel [MJ/FU]	2.615	10.706
- CO_2eq emissions [g/FU]	217	302
- CER [MJ/FU]	2.9	4.0
- CMR [kg/FU]	0.001	0.003
4. Cooking phase		
- energy [MJ/FU]	9	15
- cooking water [kg/FU]	10	10
- CO_2eq emissions [g/FU]	1242	1114
- CER [MJ/FU]	19.1	18.2
- CMR [kg/FU]	0.004	0.137
worst performance (value 2)	equivalent performance (value 1)	better performance (value 0)

Table 29: Results of the Regional Agency for the Environment Protection in Tuscany, Italy (ARPAT) model. (Source: Recchia et al., 2019).

	Overall Impact	Water Impact	Ecosystem Impact	Dose [kg/ha]
Pesticides for high-quality/traditional pasta				
Azoxystrobin	2	2	2	0.22
Cioquintocet-mexyl	2	2	2	0.02
Cyproconazole	3	3	2	0.09
Pinoxaden	2	1	2	0.09
Procloraz	2 2	2 2	2 2	0.35 0.36
Propiconazole	2	2	2	0.09
Tetraconazole	2	2	2	0.06
Thifensulfuron-methyl	1	2	1	0.31
Tribenuron-methyl	2	2	1	0.31
	2.83	3.05	2.52	1.90
Pesticides for the conventional pasta				
Tribenuron-methyl	2	2	1	1.23
Pinoxaden	2	1	2	0.18
Propiconazole	2	2	2	0.30
Azoxystrobin	2	2	2	0.50
	4.40	4.22	3.17	2.20

Looking at the transport phase, the conventional pasta chain appears to be better organized in terms of logistics and characteristics of the means of transport, even if the higher distances to be covered determine significant impacts in terms of atmospheric pollution and noise. No significant differences have been observed in the cooking phase, which, however, turned out to have a considerable impact on the whole process. In fact, it can be observed that the higher average energetic consumption for the conventional pasta is balanced by the advantages due to higher crop yields and production plants efficiencies, as illustrated in Table 28. A deeper insight into LCA results shows how the high-quality pasta achieved an overall better performance than the conventional one (1706 vs. 1765 g CO_2eq/FU; 13.7 vs. 14.3 MJ/FU; 109 vs. 126 μgPO4−/FU), substantially due to the transport phase.

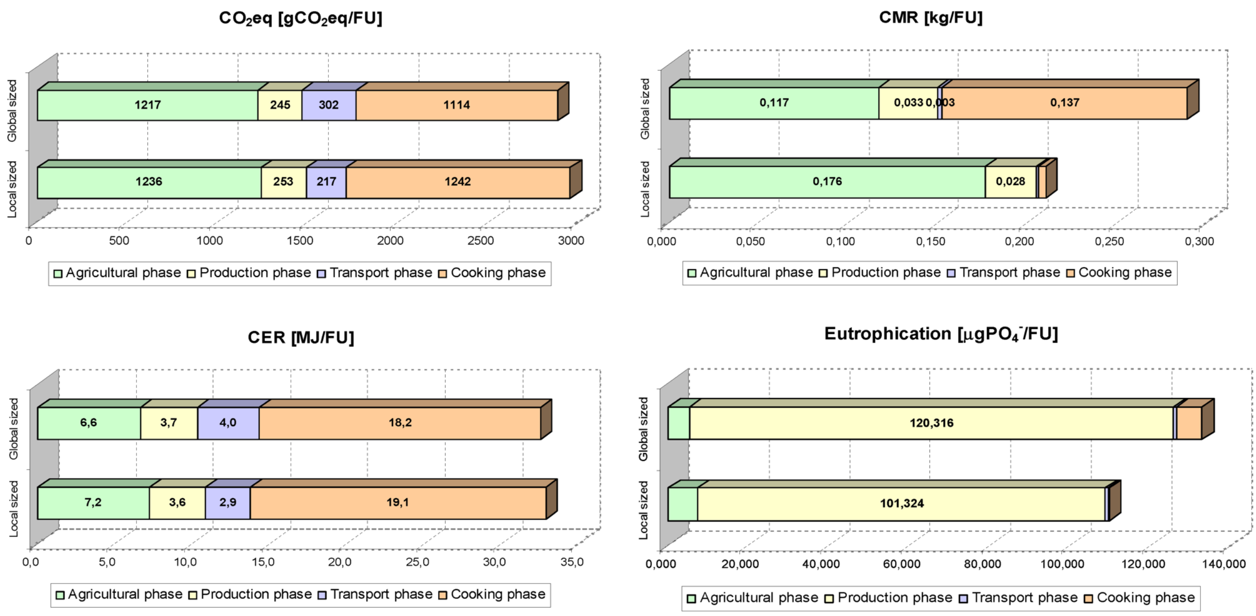

Fig 19 Life Cycle Assessment (LCA) results for high-quality/local and conventional/global pasta chains. (Source: Recchia et al., 2019).

Fig 19 reports detailed LCA results for the two chains. In particular, looking at the CO_2eq emissions, the main critical phases are represented by wheat cultivation and domestic pasta consumption, even if, as far as the CER results are concerned, domestic cooking represents 56 to 58% of the total fossil energy consumption. Furthermore, based on the results illustrated in Table 28, it is possible to assess the environmental pressures associated with the two pasta production chains (Fig. 20).

Production chain:	High quality / traditional pasta		
A – Soil	**B – Water**	**C – Air**	**D – Resources**
- Land use (A1)	- Pollution (B4)	- Pollution (C6)	- Biotic: agrobiodiversity (D8)
- Pollution (A2)	- Abstraction or Diversion (B5)	- Noise (C7)	- Abiotic: non renewable (D9)
- Degradation (A3)		**E – Climate change** (E10)	
Production chain:	Conventional pasta		
A – Soil	**B – Water**	**C – Air**	**D – Resources**
- Land use (A1)	- Pollution (B4)	- Pollution (C6)	- Biotic: agrobiodiversity (D8)
- Pollution (A2)	- Abstraction or Diversion (B5)	- Noise (C7)	- Abiotic: non renewable (D9)
- Degradation (A3)		**E – Climate change** (E10)	
worst performance	equivalent performance	better performance	

Fig 20: Assessment of the environmental pressures for the high-quality and conventional pasta production. (Source: Recchia et al., 2019).

The high-quality pasta chain shows a better performance in terms of risk reduction for soil degradation and agrobiodiversity loss, as well as consumption of non-renewable resources; this is mainly due to the use of lower quantities of chemicals, a lower mechanization level in the agricultural phase, and the use of ancient wheats. However, the conventional pasta chain prevails in terms of a more efficient exploitation of land and water resources, due to higher yields and the use of more efficient sprayers, and also in reducing noise emitted by the overall production equipment.

7.1.4 Discussion of the Environmental Assessment and Future Improvements

Several authors have analyzed LCA of pasta production in small and medium-sized industries based in southern Italy; some of them have also analyzed the Environmental Input and Output Life Cycle Assessment (EIO-LCA) (Notarnicola et al., 2004), which uses economy intersectoral matrices to include the whole economic system in the analysis, without cut-offs or other limitations of the system borders. Results of these studies are coherent and point out that the phase with the highest impact in the production process is wheat cultivation (Notarnicola et al., 2008). In 2009, the Barilla Group produced a detailed LCA and a preliminary EPD of a 500 g unit produced in Italy and packed in a paperboard box. As a result of this analysis, gross energy requirements were of 17 MJ, out of which 4.7 MJ was used in the durum wheat production, with a Global Warming Potential (GWP) of 1.3 kg of CO_2 and a water consumption of 7.2 kg. The cooking phase turned out to be the most critical one in the whole process. The Barilla study was updated in 2012 and 2014, and extended to all Barilla processing facilities in the world, finding a number of relevant actions useful for mitigating the environmental impact of each process phase.

For CO_2eq emissions for the high-quality pasta production chain, the agricultural phase seems to be the one with the most impact. A strategy which is suggested to reduce such emissions is given by the organic farming approach, which avoids the use of the fertilizers and pesticides, with the additional opportunity of using by-products for fertilizing. As a matter of fact, the Montepaldi farm adopted organic management during 2016–2017: The same cultivars were grown with an average yield of 2623 kg/ha. The mechanization level was increased, and consequently, the fuel consumption rose to 6500 MJ/ha. Furthermore, LCA results show a better overall performance of the high-quality pasta with respect to the conventional one: 1270 vs. 1765 g CO_2eq/FU; 11.4 vs. 14.3 MJ/FU; 33 g/FU vs. 152 g/FU; 110 vs. 126 μgPO4−/FU. Moreover, these results could be even lower if by-products were used for fertilizing, due to the obtaining of “credits” in the LCA assessment. An organic certification could increase the value of the product further, and could be considered as an additional motivation for consumers. For conventional pasta production, a reduction of CO_2 emissions in the agricultural phase could be pursued, too, by identifying the minimum quantity of fertilizers which guarantees suitable yields. Furthermore, another strategy which might be interesting for producers, is to convert part of the production to organic farming.

Considering the production phase of high-quality pasta, the drying phase is the most critical in terms of energy consumption, environmental impacts, and expenditure, indicating the need for improvement. The Italian traditional high-quality pasta production is based on a low-temperature long-time (LT-LT) drying process (West et al., 2013), which is recognized as a quality parameter by consumers. However, LT-LT energy consumption could be reduced by the introduction of new technologies, such as microwaves and pulsed electric fields (De Pilli et al., 2009). Furthermore, in the Montepaldi case, the impacts due to the transport phase should be significantly reduced by avoiding raw material transportation to a mill at a greater distance, and by averting transportation of semi-finished products to a distant pasta manufacturing plant. In fact, small production companies should be independent and carry out in-house cultivation, milling, and pasta production to be competitive and assure safe products with lower environmental impacts. In addition, a proper logistic should be planned for pasta distribution. On the other hand, in the global scenario, it is assumed that big companies have already optimized logistics and transport to minimize costs; as a consequence, opportunities of reducing their associated impacts are minimal.

Concerning CO_2 emissions related to the cooking phase, it is important to highlight that part of the high-quality pasta production is dispatched to restaurants, where the use of pasta cookers allows to save up to 60% of energy and 38% of water (Fusi et al., 2016). On the contrary, domestic consumption mainly uses the conventional product, so that, in this case, the most promising energy reduction strategy consists in the improvement of household cooking practices, which can lead to savings of up to 95%. Among these, a substantial reduction of carbon footprint and operating costs in domestic cooking of pasta can be obtained by using an induction hob and a pan covered by a lid: the power rate should be initially set to the maximum level, to make the cooking water boil faster, and then to the minimum level necessary to keep constant the water temperature and allow starch gelatinization. This would allow a carbon footprint reduction of up to 670 g CO_2eq and operating cost reductions up to 0.47€ per kg of pasta (Cimini & Moresi, 2010).

7.1.5 Conclusions

The results of the present work show that the traditional pasta may cause impacts of 1.706 kg of CO_2eq emissions, 13.7 MJ of fossil energy consumption, 0.206 kg of non-renewable resource, and 109 μgPO4-/FU per each kg of dried pasta produced, while the conventional pasta production accounts for 1.765 kg of CO_2eq emissions, 14.3 MJ of fossil energy consumption, 0.152 kg of non-renewable resource, and 126 μgPO4-/FU per each kg of dried pasta produced, without considering both the distribution and domestic cooking phases.

As a consequence, the high-quality pasta chain causes environmental impacts which are comparable to those of the conventional chain. Nevertheless, following the suggestions proposed here, CO_2 emissions of the high-quality pasta production chain could be significantly reduced, obtaining significant improvements in LCA assessment, when compared to the conventional pasta production in a global scenario where margins for improvement are lower.

However, though the LCA does not highlight significant differences between the high-quality and conventional pasta production chains, the proposed integrated EIAN-LCA approach shows that the high-quality chain has a lower impact on soil degradation, agrobiodiversity losses, and on the consumption of non-renewable resources. On the other side, due to higher yields, higher equipment efficiency, and lower noise emissions of the involved machines, the conventional chain has a lower impact in terms of land use, water abstraction and/or diversion, and noise emissions.

In conclusion, the developed combined EIAN-LCA approach allows to evaluate several indicators, belonging not only to LCA standards but for an innovative and comprehensive assessment of pasta production chains. This approach appears able to suggest how to enhance the various production phases, also considering the peculiarities of the geographical and technological context, to improve the sustainability of food production.

7.2 Use of alternative source of protein to reduce the environmental impacts related to the production of bakery products

7.2.1 Insects as food: a review on risks assessments of Tenebrionidae and Gryllidae in relation to a first machines and plants development

7.2.1.1 Aim of the study

Several studies suggest that edible insects may be a viable alternative or supplement to conventional protein sources (Belluco et al., 2013; Mlcek et al., 2014; Payne et al., 2016; Schlüter et al., 2017; Van Huis, 2017). The breeding, collecting, and processing methods are able to influence safety, nutritional values and technological performances of whole and powdered insects (De Gier & Verhoeckx, 2018). Insect eating is estimated to be regularly practiced by at least 2 billion people worldwide (FAO, 2014). The most commonly eaten insect groups are beetles, caterpillars, wasps, bees, ants, grass- hoppers, locusts, crickets, cicadas, termites, dragonflies, and flies (Van Huis et al., 2013; Van Huis, 2017). However, *Tenebrionidae* and *Gryllidae* families are seen as more suitable for consumption and processing (Marberg et al., 2017).

In most Western countries, consumer acceptance of entomophagy is still limited by disgust (appearance, taste, sense of dirtiness and danger perception) (Cicatiello et al., 2016; Van Huis et al., 2013; Sogari, 2015). Nonetheless, even in these regions, insect rearing is increasing and more and more insect-based food products are being studied (Fasolato et al., 2018; Osimani et al., 2018). The potential benefits of insect consumption include their nutritional properties: most insects are rich in high quality proteins (with an essential amino acid score ranging from 46 to 96%), good quality lipids (rich in alpha-linolenic acid and linoleic acid), vitamins, minerals (such as calcium, iron, and zinc) and fiber (due to the presence of chitin) (Belluco et al., 2013; Micek et al., 2014; Payne et al., 2016; Schlüter et al., 2017). These nutritional benefits are well-known and pursued in developing countries, where for a long time, the combination of the richest availability of edible insects and the presence of malnutrition have determined their largely diffused consumption (Garofalo et al., 2017; Klunder et al., 2012). However, it is important to highlight that the nutritional value of insects varies significantly among species and development stages.

Apart from nutritional qualities, the rearing of insects for food is also linked to potential economic, social, and ecological proceeds. Harvesting or farming insects can offer new employment and income-earning opportunities, including some of the poorest segments of society (FAO, 2014). Insect breeding seems to have a lower ecological footprint than livestock production: insects multiply faster, cause lower GHG and ammonia emissions, require less land area and water use, have higher feed conversion efficiency, and can potentially be bred on organic by-products (Garofalo et al., 2017; Klunder et al., 2012; Van Huis, 2017). Owing to the same properties, insects, like many food products that are rich in nutrients and moisture, also provide a favorable environment for microbial survival and growth (Garofalo et al., 2017; Klunder et al., 2012; Van Huis, 2017).

Since, as of January 2018, insects are considered as categories of food to which the European Union legislation on novel foods (Regulation (EU) 2015/2283 on novel foods) is applied, more data on the microbial, chemical, and physical safety of edible insects reared or imported in Europe are necessary (European Union, 2015). Despite this, is it important to highlight that insects are not allowed for human consumption in all the European countries. Moreover, little is known about the potential risks derived from the breeding systems, as well as those potentially derived from the transformation processes (in particular physical and allergenic risks for food chain operators and consumers).

In fact, this systematic review of the existing bibliography highlights the lack of scientific articles and reviews regarding machines, plants, and integrated supply chain approaches regarding the production chain of insects for food, thus motivating this research (Cappelli et al., 2020e). For this reason, the aim of this review is to summarize the current knowledge related to the potential microbial, chemical, physical, and allergenic risks related to the breeding, transformation, and consumption of edible insects for food (Cappelli et al., 2020e).

7.2.1.2 Search strategy

The review considers the EFSA scientific opinion (EFSA, 2015) as the starting point, which is why the years from 2016 onwards (till begin of 2019 included) have been chosen as a time frame. Three databases were explored by means of ad hoc search strings: Science direct, PubMed, and Web of Science. For the microbiological, chemical, and physical risks, a unique search string was used for all the databases, while for the risk related to allergenicity, two different search strings were used: one for Science direct and one for PubMed and Web of Science. The search strings used are as follows:

- "Food AND (Insect* OR "novel food*" OR mealworm* OR cricket* OR entomophagy OR "edible insect*") AND (microbiota OR "microb* community" OR "microb* count*" OR "microb* load" OR "microb* risk" OR "microb* hazard" OR "microb* saf*" OR "food safety")" for microbiological risk;

- "Food AND (Insect* OR "novel food*" OR mealworm* OR cricket* OR entomophagy OR "edible insect*") AND ("chemical risk*" OR "chemical hazard*" OR "chemical safety" OR radionuclide* OR arsenic OR cadmium OR copper OR zinc OR chrome OR toxin*)" for chemical risk;

- "Food AND (Insect* OR "novel food*" OR mealworm* OR cricket* OR entomophagy OR "edible insect*") AND ("physical risk*" OR "physical hazard*" OR "physical safety" OR "foreign bod*" OR "breeding substrate*" OR vegetable* OR ran OR flour*)" for physical risk;

- "Food AND (Insect* OR "novel food*" OR mealworm* OR cricket* OR entomophagy OR "edible insect*") AND (allergen*)" for allergenicity risk on Science direct and "Food AND (Insect* OR "novel food*" OR mealworm* OR cricket* OR entomophagy OR "edible insect*") AND (allerg*)" on PubMed and Web of Science.

All articles considering insects belonging to the families of *Tenebrio molitor* and *Acheta domesticus*, *Tenebrionidae* and *Gryllidae*, respectively, were included. Among all insects species, these families are seen as more suitable for consumption and processing (Marberg et al., 2017). No language restriction was imposed. No restriction regarding the publication status was enforced. All duplications have been excluded from the obtained results. In addition to the studies that mainly deal with the types of risk mentioned above, those in which they are treated in a non-exclusive but exhaustive manner were also included. However, despite the EFSA report (EFSA, 2015) mentioned other kinds of risk (such as hormones), according to the obtained results the authors decided to discuss only microbiological, chemical, physical, and allergenic risks, to which a great amount of literature was found. The search results were first screened by title and abstract reading (excluding, at this step, the articles in the form of abstract and/or index), and then by full text reading.

7.2.1.3 Main findings regarding the risks related to insects consumption

7.2.1.3.1 Results of the systematic review

A total of 4593 results were obtained: 1871 for microbiological risk, 639 for chemical risk, 660 for physical risk and 1423 for allergenicity risk. After removing duplicates within each database, screening by title and abstract reading, screening by full text reading, and removing duplicates between the three databases, 77 were selected as the sum of the results of all the search strings. By removing the articles in common between the results of the different search strings, the items selected were brought down to 64. Of these 64, three were systematic reviews, 14 were other forms of review, eight were book chapters, one was an epidemiological retrospective cohort study, and 38 were descriptive studies by laboratory researchers (32 on contaminants in insect samples and six on patients' sera). Flow charts developed according to PRISMA statement (Moher et al., 2009) related to microbiological (Fig. 21a), chemical (Fig. 21b), physical (Fig. 21c), and allergenic risks (Fig. 21d) are shown in Fig. 21.

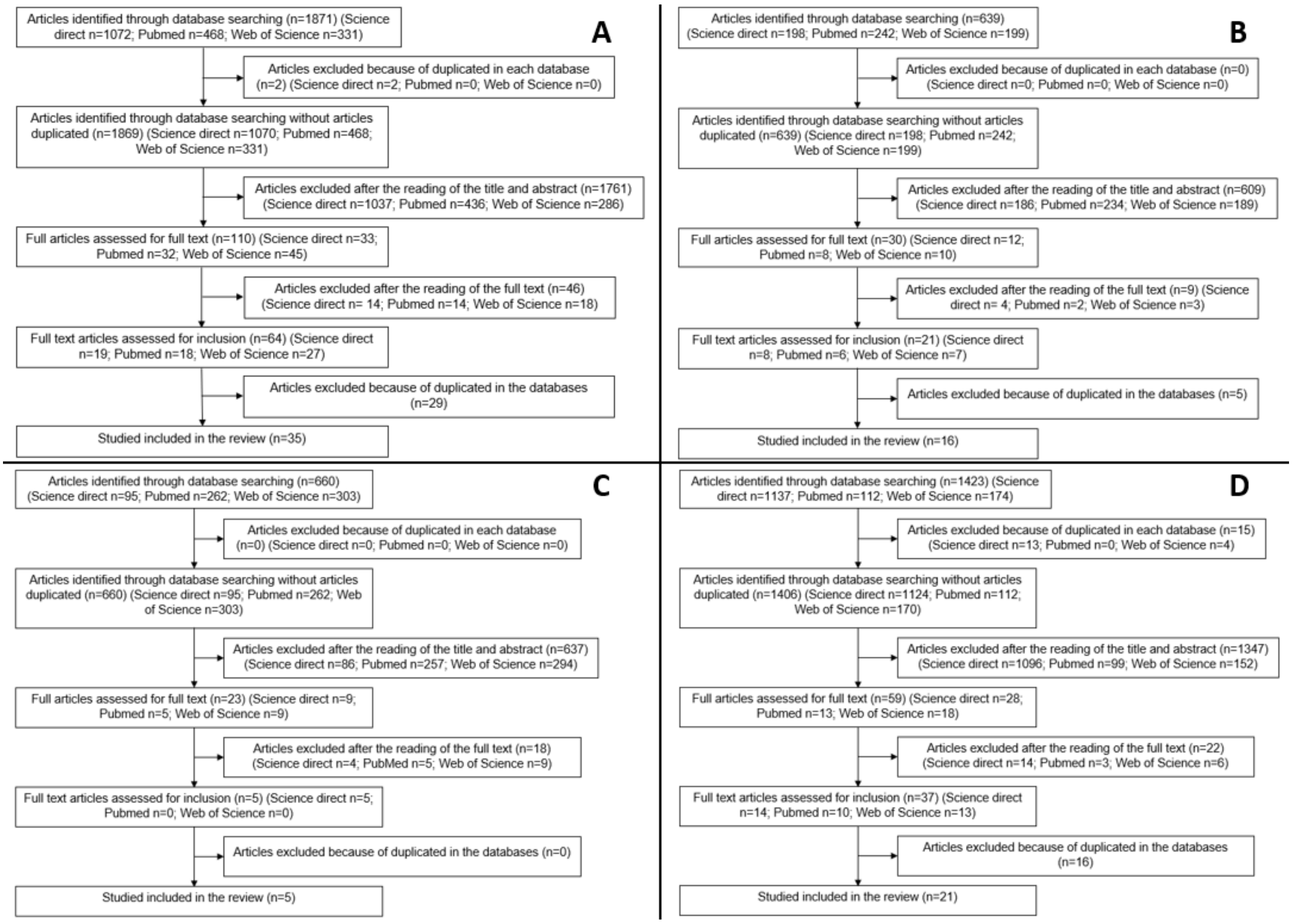

Fig 21: Flow charts regarding the selection of papers on microbiological (a), chemical (b), physical (c), and allergenic (d) risks obtained according to the results of the systematic review. (Source: Cappelli et al., 2020e).

7.2.1.3.2 Microbiological risks

The bacterial communities found in mealworms are composed of Actinobacteria, Firmicutes, Proteobacteria, and Tenericutes (Garofalo et al., 2017), while those present in house crickets (both in whole and in powder form) are Proteobacteria, Firmicutes, and Bacteroidetes (Garofalo et al., 2017). In a study by Garofalo et al. (2017), the microbiological profile of certain insect species has been examined, including mealworm and house cricket. The total mesophilic aerobic count was the highest in powdered cricket (4.80 ± 0.06 log cfu g^{-1}) and the lowest in mealworm larvae (< 2.00 log cfu g^{-1}). Whole crickets were characterized by the higher Lactic Acid Bacteria (LAB) counts, while the lowest values were recorded in mealworm larvae and powdered crickets.

Yeast counts were the highest in whole crickets and molds were the highest in powdered crickets and lowest in whole crickets. The Enterobacteriaceae count varied in the selected studies: high in some (Osimani et al., 2018) while in others low, and, in two cases, below the limit of detection (Fasolato et al., 2018; Garofalo et al., 2017). However, a heat treatment (e.g. blanching or boiling) reduces the Enterobacteriaceae count without sporulating bacteria and bacterial spores (De Castro et al., 2018; Gallo, 2018; Liu & Zhao, 2018; Van der Fels-Klerx, et al., 2018; Van Huis, 2017). Salmonella spp. and Listeria monocytogenes were not detected in any of the selected studies (Garofalo et al., 2017; Grabowski & Klein, 2017; Osimani et al., 2018; Vandeweyer et al., 2017a; Vandeweyer et al., 2017b; Wynants et al., 2018). Despite the latter two were not detected in the selected studies, this does not mean that insects are safe and free from these pathogens. In fact, their monitoring still remains important for consumers' safety (Cappelli et al., 2020e).

Vandeweyer et al. (2017b) studied the effect of refrigerated storage and microwave drying, after a blanching treatment, on microbial load in yellow mealworm larvae. It was observed that, except for spores, blanching leads to a significant reduction of all microbial counts at any time interval. Except for one batch (in which a slight growth of mesophilic and psychrotrophic organisms was observed), no microbial growth occurred during refrigerated storage. After blanching, the chilled conditions allowed mealworms to be stored for at least six days without spoilage, as the numbers remained well below the level considered for food spoilage (7 log cfu/g). Long drying times after blanching led to large reductions in total counts until a plateau was reached. Drying after blanching resulted in Enterobacteriaceae, LAB, yeast and molds counts below or very close to the detection limit. For bacterial endospore counts, blanching and drying resulted in lower reductions (between 0.3 and 1.0 log cfu/g).

Regarding the fungal biota, Vandeweyer et al. (2017b) report a difference between mealworms and crickets: molds were dominant in mealworm larvae and yeasts in crickets. Fungi present in feed may contaminate insects and the major risk concerns those able to produce mycotoxins as Aspergillus, Penicillium, Mucor and Rhizopus, as insects are able to metabolize and accumulate these compounds (Van der Spiegel, 2016). However, good hygiene procedures during the production chain can reduce the risk of fungi in insects (Gallo, 2018). Moreover, a blanching treatment significantly reduces the presence of fungi (Vandeweyer et al., 2017b; Wynants et al., 2018). Viruses that are pathogenic for insects are not a risk for humans because they are specific to invertebrates and because of the genetic difference between human and insect. However, although there is no evidence of pathogenic viruses in insect, they may be able to act as mechanical vectors for human pathogenic viruses (Gallo, 2018; Van der Fels-Klerx et al., 2018).

EFSA (EFSA, 2015) reports that lower risks regarding house cricket consumption are those related to viruses, prions, fungi, and parasites. There is no evidence of the presence of parasites in bred insects, but it can be assumed that this risk can be eliminated under controlled breeding conditions: in fact, in controlled breeding, the elements needed to complete their lifecycle are lacking, and processing (freezing and cooking) can contribute in the elimination of this risk (Gallo, 2018).The prions of insects have not been described and no diseases caused by them have ever been observed (Grau et al., 2017; Van der Fels-Klerx et al., 2018). The risk related to prions is linked to the substrate: if the substrate is contaminated, the insects may be able to act as mechanical vectors in their transmission to humans (Gallo, 2018; Van der Fels-Klerx et al., 2018; Van der Spiegel, 2016).

7.2.1.3.3 Chemical risks

Chemical contaminants, like toxins, can be absorbed by insects from the feed or the insects themselves can synthesize these compounds (Schlüter et al., 2016). It is reported that darkling beetles, from the *Tenebrionidae* family, produce quinones and alkanes, but the risk arising from these substances needs to be better investigated (Schlüter et al., 2016). However, it is important to highlight that darkling beetles are not usually consumed as food. In a study by Poma et al. (2017), the levels of different chemical contaminants in different insects and insect products were analyzed. Poly-Chlorinated Biphenyl (PCB) levels were higher in cricket croquettes (1712 pg/g ww) than in Alphitobius diaperinus-based food (783 and 156 pg/g ww), Alphitobius diaperinus larvae (33.8pg/g ww) and *Tenebrio molitor* larvae (26.5pg/g ww). In the products mentioned, the level of Organo-Chlorine Pesticide (OCP) was higher in *Tenebrio molitor* larvae (156 pg/g ww). Poly-

Brominated Di-phenyl Ether (PBDE) and Halogenated Flame Retardant (HFR) were below the limit of quantification, except for one Alphitobius diaperinus-based food (16.2pg/g ww). A total of six Phosphorous Flame Retardants (PFRs) were detected and their concentration in samples were higher than the other compounds analyzed, which might be due to their use in treatment processes and packaging. Zinc was the most abundant metal in samples, followed by copper (Poma et al., 2017).

In a study on contaminated substrates with different heavy metal levels, it was observed that *Tenebrio molitor* larvae can accumulate arsenic but not cadmium and lead (Van der Fels-Klerx et al., 2018). Regarding mycotoxin contamination, the results of Bosch, van der Fels-Klerx, De Rijk, and Ooninc x (2017) suggest that aflatoxin B1 is rapidly catabolized and excreted by yellow mealworm larvae, either by conversion into aflatoxin M1 or simply as aflatoxin B1. This highlights a significant risk related to bioaccumulation. Moreover, the study reported by Van Broekhoven et al. (2017) shows that *Tenebrio molitor* larvae are able to metabolize deoxynivalenol because the remaining fraction of this compound, i.e. the portion excreted through faeces, has not been detected. Cooking or digestion can remove or deactivate some chemical hazards (Liu & Zhao, 2018). However, collecting insects for the production of food and food ingredients must be done in such a way as to prevent or minimize the accumulation of toxins, drugs, and antinutrients coming from outside (Schlüter et al., 2016). In particular, the most critical steps regard the breeding phase, which is crucial to control the chemical risk, and the transformation process.

7.2.1.3.4 Physical risks

In none of the selected articles is the physical risk treated specifically for yellow mealworm larvae or house cricket. Only general information is given for all insects. Choking is the greatest physical hazard associated with insects consumption: in fact, the insect body is inhomogeneous because it has appendages like spines or cuticles, which make insects a foreign texture to those with which Western palates are accustomed (it is particularly hazardous for elderly people and children, owing to greater difficulty in ingestion) (Marone, 2016). For this reason, powdered insects are considered safer than whole insects and it is also the form that mostly facilitates consumer acceptance (Marone, 2016). The control of physical hazards when the risk is identified as a part or an ingredient of the food, in our case represented by the whole insect or a part of it, is particularly difficult; the best way to prevent it is to follow good manufacturing practices (e.g. IPIFF guide on Good Hygiene Practices) and increase the awareness of employees in controlling all the steps of the food chain. However, according to the degree of risk exposure, the adoption of personal protective equipment (PPE), such as filtering facepiece respirator, could protect the workers during the processing phases.

7.2.1.3.5 Allergenic risks

Based on the number of results obtained and the fact that most of the articles confirm the likelihood of developing food allergy to insects, it can be stated that the risk of allergic reactions related to the consumption of insects is significant. People allergic to crustaceans and house dust mites can develop allergic reactions to insects due to the close taxonomic relationship between insects and arthropods, which helps to predict allergens (Downs et al., 2016). The major allergens that cause cross-reactivity in insects are tropomyosin and arginine kinase, but there are also other allergens such as sarcoplasmic calcium-binding protein, myosin light chain, troponin C, sarcoplasmic endoreticulum calcium ATPase, hemocyanin, and phospholipase (Downs et al., 2016).

In a study by Broekman et al. (2017), it has been observed that mealworm breeders (both domestic and professional) develop inhalant allergy and food allergy to this insect, and binding IgE were found towards allergens as tropomyosin, arginine kinase, myosin light chains, and myosin heavy chains. In shrimp-allergic patients, mealworm consumption shows different symptoms like oral allergy, urticaria, nausea, abdominal cramping, vomiting, and dyspnea (Broekman et al., 2017). More in general, insect food allergy can occur in different ways (from a mild localized reaction to anaphylactic shock) and symptoms are subdivided into cutaneous, gastrointestinal, and respiratory.

Regarding cricket allergenicity, Hall et al. (2018) studied cross-reactivity between human shrimp-allergic sera and tropomyosin in crickets (Gryllodes sigillatus) at different degrees of hydrolysis: cricket protein hydrolysates with a degree of hydrolysis of 60–85% had the lowest reactivity to tropomyosin compared to those with a degree of hydrolysis of 15–50% and to the unhydrolyzed cricket. Processing can affect insect allergenicity. In fact, tropomyosin allergenicity seems to be decreased in fried samples and disappeared in fried and in vitro digested samples (Van Broekhoven et al., 2017). Apart from proteins, there is also an allergen of insects among carbohydrates, namely chitin. Chitin is a fiber present in the exoskeleton of insects (also in crustaceans) (Van der Spiegel, 2016). The effect of processing on allergens can be understood from studies devoted to closely related arthropod species, as there is not much information regarding insect-specific allergens (Downs et al., 2016). However, data from recent literature about the effect of thermal treatment are contradictory because highlight that the allergenicity may decrease, increase, or remain the same (Broekman et al., 2017; De Gier & Verhoeckx, 2018; Van Broekhoven et al., 2017). Contradictory results may be explained by the fact that processing changes the solubility of proteins (De Gier & Verhoeckx, 2018). Although digestion has an effect on food allergenicity due to the acidic environment, protein degradation does not always occur completely and sometimes does not occur at all (De Gier & Verhoeckx, 2018).

7.2.1.4 *Strategies to reduce the risks related to the consumption of insects as food*

From the selected studies chosen by means of the systematic process of review emerges the exigence of developing machines and processes to reduce the microbiological, chemical, physical, and allergenic risks related to emerging production chain of insects for food. This is necessary in order to lay the foundations for the development of a specifically designed supply chain of insects for food, which can guarantee the safety and health for workers and consumers.

7.2.1.4.1 Strategies for reducing the microbiological risks

The microbiological content of *Tenebrio molitor* and *Acheta domesticus* (fresh, processed, or stored) was evaluated in the study by Klunder et al. (2012), along with the effect of certain processes on it. The effect of boiling was observed to completely eliminate Enterobacteriaceae after 5 min of treatment. In crickets, however, prolonging the boiling time to 10 min does not affect the level of the remaining bacteria (Klunder et al., 2012). Boiling and cooking under vacuum were seen to be the most efficient treatments for reducing the microbial load in mealworms (Caparros Megido et al., 2018). Based on the obtained results, Klunder et al. (2012) recommend applying boiling before storing insects in the refrigerator.

Another heat treatment that can be applied to insects is blanching. In the study by Vandeweyer et al. (2017b), it is shown to lead to a significant reduction of microbial counts. Particularly for blanching times of 10, 20, and 40 s, log reductions for total counts of 4.4, 6.4, and 5.6 log cycles were observed (no relationship between the magnitude of the reduction and blanching times was observed). Blanching followed by refrigerated storage can preserve mealworms without spoilage for at least six days (Vandeweyer et al., 2017b). In the same study, it was observed that a blanching treatment followed by microwave drying represents a pasteurization treatment because of the killing of vegetative cells but not of the spores. Moreover, blanching preceded by roasting leads to a strong reduction in the number of Enterobacteriaceae (FASFC, 2014), although Klunder et al. (2012) observe that roasting alone is not able to completely eliminate Enterobacteriaceae. No treatment was found to be capable of significantly reducing the count of sporulating bacteria and bacterial spores: in the reviewed studies, no one was able to eliminate them with heat treatment.

After heat treatments, insects may be frozen or freeze-dried: these two treatments are applied to obtain an extension of the shelf life of the product, but it was observed in a preliminary study that they barely influence the total aerobic bacterial count of the insects (FASFC, 2014). The crushing of the larvae leads to the release of microorganisms contained in the intestine and to their distribution across the whole insect; this leads to an increase in viable bacteria count (Klunder et al., 2012). According to Rumpold and Schlüter (2013), a crushing step before thermal treatment (boiling or roasting) does not improve the efficacy but results in a higher microbial content in comparison with the whole insect. However, confirmation studies which test several time-temperature ratios and different methods of heating application (e.g. direct and indirect heating) are necessary to validate this statement. One possibility to reduce the contamination resulting from the microbiota of insect gut, is to adopt a starvation period before insect harvest. However, the effect of a starvation period of 48 h has been studied; there is no significant effect of reducing the microbial content of insect gut (Wynants et al., 2017). Further studies are needed to clarify the effect of starvation in edible insects.

Fermentation of insects is also documented. It is used to increase the shelf-life and microbial safety of ground insects (FASFC, 2014). It was observed that fermentation deactivates Enterobacteriaceae and maintains spore-forming bacteria under 103 cfu/g (Klunder et al., 2012). During fermentation, spores were unable to germinate and grow (Klunder et al., 2012). Borremans et al. (2018) studied fermentation of insects, specifically in *Tenebrio molitor* larvae; their results suggest that to obtain rapid acidification with the aim to inhibit undesirable microorganisms, a starter culture should be added. In this study, marination of insects was also investigated; it was observed that it can prolongs the shelf life of fresh mealworms for at least seven days due to a reduction of pH which inhibits microbial growth during storage (Borremans et al., 2018).

Cold atmospheric pressure plasma is a treatment that can be applied without the use of heat. Buβler et al. (2016) studied the effect of cold atmospheric plasma on the microbial load of *Tenebrio molitor* flour. It led to a reduction in the microbial content, the magnitude of which increased with the prolongation of treatment time: the initial microbial load of 7.72 log10 cfu/g was reduced to 7.10 log10 cfu/g after 1 min and to 4.73 log10 cfu/g after 15 min of treatment (Buβler et al., 2016). According to present knowledge, the most interesting strategies to reduce the microbial contamination would include the addition of blanching machine in the production chain, followed by refrigeration in the case of whole insect conservation, or microwave treatment before processing. However, after the blanching and/or microwaves treatments, which reduce the microbial load, the fermentation with selected strains of yeasts and LAB might significantly reduce the microbiological risk, exalting the efficacy and efficiency of the blanching and/or microwaves treatments.

7.2.1.4.2 Strategies for reducing the chemical risks

Regarding chemical contaminants, EFSA (2015) reports that processing conditions (e.g. heating or freeze-drying) have only minimal effect on them. The best way to control the levels of chemical contaminants is fractionation because they are accumulated in specific insect fractions (EFSA, 2015). For the prevention of chemical risk, it is important to control insect breeding substrates, feed, and the environment in which insects are bred, because of the ability of insects to accumulate chemical contaminants. In addition to breeding, a check could be applied for the presence of chemical contaminants during the process. For the rapid detection of chemical contamination, several methods can be used: immunodetection (which is based on the interaction between an antigen and an antibody, it is used in pesticide detection, for example), chemistry method (in presence of metallic ion, organic agrochemical of phosphorous hydrolyze giving rise to a hydrolysis product that leads to color change) and biosensor (the concentration of the contaminant is converted into a measurable electrical signal) (Tang et al., 2009). For heavy metal analysis, the common techniques employed are different types of spectrometry; however, these methods require a laborious phase of sample preparation and are not possible in time restrictions.

Recently, nanotechnology is often used to provide sensors for heavy metal detection in the environment, such as fluorescent sensors, plasmodic sensors, surface-enhanced raman scattering (SERS) sensors, optofluidic sensors, electrochemical sensors, field-effect transistor (FET) sensors (Li et al., 2013). Due to the fact that the accumulation of heavy metals in insects comes mainly from feed, these sensors could be used for heavy metal detection in the breeding environment. Apart from feed and breeding, control should be maintained throughout the production process because chemical contaminants could be introduced during unit operations, for example migration from the machines used. From this point of view, in the choice of the machines to be included in the production process, the evaluation of the usability of the machines is important. An example of the migration of chemical contaminants in food during a unit operation, specifically the grinding, is reported by Yahaya et al. (2012): in ground food samples, an increase in iron content was observed, so it was recommended to subject the grinding discs to heat treatment to minimize metal contaminants in ground foods. In accordance with the findings, possible strategies intended to reduce the chemical risk could start by preventive approach in the breeding phase, followed by careful fractionation and finally rapid detection strategies during the critical control points of the process with nanosensors.

7.2.1.4.3 Strategies for reducing the physical risks

As previously mentioned, the major physical hazard present in insects is choking: the way to limit this risk is to grind insects (Marone, 2016). For an optimal control of physical risks, critical points should be identified throughout the production process. In particular, the HACCP system should be focused on the most critical phases (sorting, grinding, and packaging) to guarantee safe working conditions and product free from foreign bodies. Moreover, unit operations are crucial so it would be advisable to use machines that prevent the introduction of foreign bodies, such as closed grinders, with significant reduction in the inhalation risk for the workers. To verify the good functioning of the most critical unit operations, of the HACCP plan and of the identified critical points, the installation of instruments capable of detecting the presence of foreign bodies during the process and in the product might be useful. The presence of foreign bodies such as metals and glass can be verified by installing metal detectors or X-ray machines.

The operators involved in the production must follow correct personal hygiene practices (in particular the good manufacturing practices in force (e.g. IPIFF guide on Good Hygiene Practices)) and wear suitable clothing and protections to prevent the introduction of foreign bodies and the risk of inhalation. In agreement with the scarceness of data collected, with the utilization of an effective insect-sorting machine, it is possible to effectively reduce the risk of foreign-body contamination in the product. An important focus should regard the physical risk related to the workers' activities. In particular, the selection of machines optimized in ergonomics and usability terms, as automated as possible, would reduce the cutting and contusion risks along the production chain of insects for food. Furthermore, in order to guarantee workers safety, another important focus might regard the adoption of specific PPE, such as filtering facepiece respirator, which could protect the workers from in- halation risk during the processing phases.

7.2.1.4.4 Strategies for reducing the allergenic risks

As previously reported, there are in literature contradictory data about the effect of thermal treatment on the food allergenicity of insects (De Gier & Verhoeckx, 2018). In fact, EFSA (EFSA, 2015) also reported that the allergenicity of certain compounds can be triggered or blocked through heat treatment that alters the structure of proteins (Fernandez- Cassi et al., 2018). Although, the understanding of the effect of processing on insect allergens can be found in studies devoted to closely related arthropod species (Downs et al., 2016), there are some studies that investigate allergenicity. Broekman et al. (2017) were the first to study the effect of heat treatment on mealworm allergenicity: they observed that the IgE-binding capacity and IgE cross-linking functionality of mealworm allergens were not lowered by the processing, while the solubility of proteins was strongly influenced. As the heat treatment seems to increase the solubility of tropomyosin, it was hypothesized that this phenomenon happened when the bonds with proteins were broken or when the formation of soluble aggregates took place during the treatment (Broekman et al., 2017). Tropomyosin allergenicity, in the study by Van Broekhoven et al. (2017), decreased in fried samples and disappeared in fried and in vitro digested samples. On the other hand, heating causes the arginine kinase to unfold, exposing the hydrophobic amino acids and leading to the formation of larger protein aggregates that, in most cases, are insoluble (Broekman et al., 2017).

Regarding the allergenicity of crickets, in a study by Hall et al. (2018), it was observed that the enzymatic hydrolysis of proteins had an effect on tropomyosin allergenicity: in fact, cricket protein hydrolysates with a major degree of hydrolysis showed the lowest reactivity of tropomyosin. Given the consistency of information in the references, it is advisable not to encourage people who are allergic to crustaceans and house dust mite to consume insects. It is, therefore, essential that the information accompanying the product must be clear: on the label, it should be reported a warning addressed to people allergic to crustacean and house dust mites, informing them of the allergenic risk that they run in case of consumption of the product. However, frying and enzymatic hydrolysis seems to be the most interesting strategies to reduce the tropomyosin allergenicity.

Finally, further investigations are needed to assess the effect on workers' health connected with the risk of developing allergenicity due to inhalation during the grinding unit operation and in others phases of the production chain (Broekman et al., 2017). In particular, a clarification is necessary regarding the effects on workers' health to understand if single exposure is sufficient to produce damages on health or if prolonged exposure is needed.

7.2.1.5 Conclusions & future trends

The growing demand of protein food with reduced environmental impacts is currently a big challenge for food production. Insects represent a potential frontier of good quality food, rich in nutrients and easy to be produced, at cheaper costs and with possible lower environmental impact.

Nonetheless, it is fundamental for the consumers' safety to conduct prospective studies on the risks raised by them, since in the majority of the EU Countries, as well as in the Northern part of America, their use is too recent to have solid evidences on the impact on health, as well as on the effectiveness of the processes used to control the described risks.

As for all the novelties, insects will also require specific surveillance systems that can promptly record any unfavorable episode for consumers' health, which can address the private and public control to improve the safety of the whole production chains, so as to ensure safe products on the market. Many other primary studies are necessary to untie these nodes, but this review is a preliminary proof that authorized novel foods can, at least partially, represent a possible answer to the increasing demand of protein food by a population that is forecast to grow significantly and whose food demand will not be reduced in the future.

This paper suggests strategies to orient the development and improvement of specific machines for the emerging production chain of insects for food, with ameliorative effects on workers and finished products safety.

In particular, this review aims to lay down the foundations for the development of the production chain of insects for food which should be based on integrated supply chain approaches, specific self-regulations and HACCP plans, development of ad hoc machines, and defined processes flowcharts that might lead to a specific European Regulation for edible insects.

7.2.2 Assessment of the rheological properties and bread characteristics obtained by innovative protein sources (Cicer arietinum, Acheta domesticus, Tenebrio molitor): Novel food or potential improvers for wheat flour?

7.2.2.1 Aim of the study

The world's population is growing constantly, and is expected to reach 9.7 billion by 2050 (United Nations, 2015). This growth goes hand-in-hand with increased demand for protein, exacerbating environmental pressure. There is therefore a need for food production with reduced environmental impact in order to limit Green House Gas (GHG) emissions, lower the energy consumptions, and optimize land use. All of these factors point to the need for alternative sources of protein.

Currently, research trends and food innovation are focused on sustainable vegetable and animal protein sources. Among sources of animal protein, insects are a potential solution for the food industry. Insects are a traditional part of the diet of two billion people and 1900 species are reported to be regularly consumed (Van Huis et al., 2013). While in Asia, Africa and South America entomophagy has long been part of the tradition of certain cultures, it has only recently been considered in the Western world. The European Union took a step forward in this direction with the entry into force of Regulation (EU) 2015/2283 (European Union, 2015), which recognizes insects as a novel food.

The use of insects in the food industry has several positive benefits: firstly, an increase in high-quality protein and nutrients in foods (Rumpold & Schlüter, 2013); secondly, high feed conversion efficiency, which significantly reduces rearing costs (Collavo et al., 2005); thirdly, the reproduction rate is high compared to other animal protein sources (Van Huis et al., 2013); and finally, reduced water consumption and GHG emissions (Oonincx & De Boer, 2012). Neophobia, disgust and non-acceptance are identified as the major obstacles for the consumption of insects as food in Western countries (Megido et al., 2016). However, studies have demonstrated that food neophobia and disgust decrease if insects are added in an invisible form into products food such as flour, or if they are associated with known flavors (Megido et al., 2016). As a result, a few innovative studies of insect-based doughs, paste, and products (such as extruded snacks, bread, and meat analogues) are reported in literature.

Legumes are another interesting source of protein as they contain high amounts of essential amino acids such as lysine, threonine, valine, and tryptophan. Cereals (unlike legumes) are rich in sulfur amino acids, and their combination creates a protein with high biological value. Chickpeas, in particular, have high omega 3 fatty acid and lecithin content and can help to control blood pressure, increase HDL cholesterol, and reduce LDL cholesterol (Ranalli et al., 2018). Furthermore, chickpea cultivation reduces the use of nitrogen fertilizers (due to their nitrogen-fixing capacity), with positive impacts for sustainable agriculture and on subsequent crops (Carranca et al., 1999). As a consequence, the literature reports the results of several studies of foods enriched with chickpea flour, such as cakes and bread.

Recently, the consumers interest in food quality and sustainable production processes had led to the revival of ancient wheats which contribute to the safeguarding of environment (Recchia et al., 2019) and of crop biodiversity (Cappelli et al., 2018). Despite their high nutritional content, ancient grain flours have poor technological properties, and improvements need to be found.

This research investigates the effects of the substitution of wheat flour with alternative sources of protein (mealworm, cricket and chickpea) on the rheological properties of doughs and bread characteristics (Cappelli et al., 2020h). The first aim was to evaluate which alternative protein source performed better both from a technological and nutritional point of view (Cappelli et al., 2020h). The second aim was to compare dough rheology and bread characteristics for breads made with 100% refined wheat flour to those made with mealworm, cricket and chickpea flours (Cappelli et al., 2020h).

7.2.2.2 *Materials and methods*

7.2.2.2.1 Raw materials and flour preparation

Pure refined white flour (Verna) was kindly provided by New.co.pan Ltd. (Florence, Italy). Cricket flour (*Acheta domesticus L.*) was provided by DL Novel Food Ltd. (Cuneo, Italy). Mealworm flour (*Tenebrio molitor L.*) was kindly supplied by Microvita Ltd. (Bologna, Italy). Finally, chickpea flour (*Cicer arietinum L.*) produced by Sarchio ltd. (Modena, Italy) was purchased in a local supermarket. Table 30 presents the proportions of flours used in trials. Salt (Chantesel Ltd.), brewer's yeast (Zeus, Zeus IBA Ltd., Florence, Italy) and water (Sant'Anna, Fonti di Vinadio Ltd.) were purchased in a local supermarket.

Table 30: Substitution rates for the preparation of tested flours. (Source: Cappelli et al., 2020h).

Samples	Refined wheat flour (%)	Mealworm flour (%)	Chickpea flour (%)	Cricket flour (%)
Control (100% wheat)	100	0	0	0
Mealworm flour 5%	95	5	0	0
Mealworm flour 10%	90	10	0	0
Mealworm flour 15%	85	15	0	0
Chickpea flour 5%	95	0	5	0
Chickpea flour 10%	90	0	10	0
Chickpea flour 15%	85	0	15	0
Cricket flour 5%	95	0	0	5
Cricket flour 10%	90	0	0	10
Cricket flour 15%	85	0	0	15

7.2.2.2.2 Experimental design

The first aim of the experiment was to assess differences between the rheological properties of doughs and bread characteristics as function of two factors: flour type and percentage substitution (Cappelli et al., 2020h). Regarding flour type, three types were tested: mealworm, cricket, and chickpea. With respect to the substitution percentage, three levels were tested: 5%, 10%, and 15%. A full factorial experimental design was developed in order to evaluate which of the tested sources of protein performed better (Cappelli et al., 2020h).

The second aim of this work was to investigate the performance of substituted flours compared to refined wheat flour in terms of dough rheology and bread characteristics. Consequently, the results of the rheological and bread tests were compared with the results for the control sample (100% refined wheat flour) (Cappelli et al., 2020h). All tests were carried out in three replicates.

7.2.2.2.3 Flour characterization

The analysis of substituted wheat flours was carried out by the Analytical Food Laboratory (Florence, Italy), according to AOAC approved methods (AOAC, 2005). In particular, crude protein (Kjeldahl method, AOAC 920.87) using the N*6.25 conversion rate, fat content (AOAC 922.06), and ashes (AOAC 923.03) were determined (AOAC, 2005). Carbohydrates were determined by the difference [100 − (protein + fat + ash)], consistently with other authors (González et al., 2019). Finally, flour moisture was determined by gravimetry at 105 °C until constant weight was achieved.

7.2.2.2.4 Rheological assessment of dough

Rheological properties were evaluated with a farinograph (Brabender, Duisburg, Germany) according to the standard method (AACC, 2000), with a Chopin NG alveograph, associated with an alveolink integrator–recorder (Chopin Technologies, Villeneuve-La-Garenne, France), in accordance with the standard protocol (ISO, 2008). Regarding farinograph assays, the ICC method ((ICC 115/1) International Association for Cereal Chemistry, 1992) was adopted: water absorption (WA); dough development time (DDT); dough stability (S); and degree of softening (DS) were assessed in three replicates. No salt was added in farinograph trials.

Furthermore, water absorption percentages recorded in farinograph trials were used to identify the optimum amount of water to be added in the breadmaking process, following earlier work (Cappelli et al., 2019a; Cappelli et al., 2020f). Concerning the dough rheology assessment with the Chopin alveograph, dough tenacity (P), dough extensibility (L), deformation energy (W), curve configuration ratio (P/L), and the index of swelling (G) were evaluated in three replicates.

7.2.2.2.5 Breadmaking process

The straight dough method was applied to make the bread. Mixing of the ingredients, dough formation, resting, leavening with fresh brewer's yeast, and the baking process were carried out with a bread machine (Pain Dorè, Moulinex, Ecully, France). The following recipe was used: 310 g of flour, 13 g of brewer's yeast, 9 g of salt and a variable amount of water (Table 32) according to the water absorption percentages recorded in farinograph trials (Cappelli et al., 2019a). The breadmaking procedure started with flour preparation, following the substitution proportions reported in Table 30, and with homogenization of samples. Kneading was performed at 110 RPM and 20 °C for 15 min. Then, the dough was left to rest and leaven at 40 °C for 1 h and 33 min. Finally, it was baked at 180 °C for 48 min.

7.2.2.2.6 Bread characterization

In accordance with other authors (Parenti et al., 2019), the standard millet displacement method (AACC, 2000) was used for the assessment of bread volume (L). Crumb density (g/ml) was determined according to the AACC Method 10–05.01 (AACC, 2000) from the mass/volume ratio, as reported by other authors (Le Bleis et al., 2015; Cappelli et al., 2019a). Crumb and crust moisture (g/100 g) were measured by gravimetry at 105 °C until a constant weight was achieved.

7.2.2.2.7 Statistical analyses

The effect of flour type and percentage of substitution were assessed using a fixed effects model, as described by Montgomery (2017). In particular, rheological and bread parameters were tested with a two-way ANOVA for flour type, percentage of substitution, and their interaction. Significance was set at $p < 0.05$. When the significance level was reached, a post-hoc Tukey HSD test was performed.

Furthermore, in order to highlight any potential ameliorative effects of substituted flours on dough rheology and bread characteristics compared to the control (100% wheat flour), the Wilcoxon–Mann–Whitney rank-sum test (WMWRST) was run (Montgomery, 2017).

Finally, the relationship between rheological properties (i.e. farinograph parameters WA, DDT, S, DS, and alveograph parameters G, P, L, W, and P/L) and flour components (protein, fat, and carbohydrates) was investigated with a multiple ordinary least square regression (MOLS) (Montgomery, 2017).

7.2.2.3 Results and discussion

The results of the flour characterization are reported in Table 31. Moreover, the results and discussions regarding dough rheological properties and bread characteristics are reported in sections below.

Table 31: Results of flours characterization and analyses (based on dry weight). (Source: Cappelli et al., 2020h).

Samples	Protein (g/100 g)	Fat (g/100 g)	Ash (g/100 g)	Carbohydrates (g/100 g)	Moisture (g/100 g)
Control (100% wheat)	13.96	1.50	0.40	84.14	15.10
Mealworm flour 5%	15.72	2.38	0.56	81.34	14.97
Mealworm flour 10%	17.49	3.25	0.72	78.55	14.83
Mealworm flour 15%	19.25	4.13	0.88	75.75	14.70
Chickpea flour 5%	14.30	1.46	0.52	83.72	14.89
Chickpea flour 10%	14.65	1.41	0.64	83.30	14.68
Chickpea flour 15%	14.99	1.37	0.76	82.88	14.46
Cricket flour 5%	16.72	1.79	0.58	80.92	14.56
Cricket flour 10%	19.48	2.07	0.75	77.70	14.02
Cricket flour 15%	22.24	2.36	0.93	74.48	13.48

7.2.2.3.1 Farinograph

Table 32 summarizes the results of farinograph tests and the two-way ANOVA.

Table 32: Results of farinograph tests and p-values assessed with the two-way ANOVA. Results are expressed as mean values of three replicates ± SD. (−) indicates no significant difference at p < 0.05. (Source: Cappelli et al., 2020h).

Samples	Water absorption (%)	Dough development time (min)	Dough stability (min)	Degree of softening (UB)
Control (100% wheat)	53.5 ± 0.44	3.69 ± 0.76	6.78 ± 0.86	61.67 ± 7.64
Mealworm flour 5%	53.0 ± 0.30	4.81 ± 0.17	7.50 ± 0.50	55.00 ± 5.00
Mealworm flour 10%	52.3 ± 0.65	4.22 ± 0.75	9.00 ± 1.15	45.00 ± 5.00
Mealworm flour 15%	51.7 ± 0.67	3.50 ± 0.17	8.50 ± 0.60	35.00 ± 13.23
Chickpea flour 5%	54.4 ± 0.79	4.22 ± 0.63	8.52 ± 0.84	51.67 ± 10.41
Chickpea flour 10%	55.1 ± 0.61	4.50 ± 0.00	7.58 ± 0.63	41.67 ± 2.89
Chickpea flour 15%	55.7 ± 0.44	4.89 ± 0.19	7.06 ± 1.25	48.33 ± 2.89
Cricket flour 5%	54.3 ± 0.58	3.69 ± 0.17	7.50 ± 1.32	50.00 ± 5.00
Cricket flour 10%	54.6 ± 0.15	4.00 ± 1.00	7.75 ± 0.66	60.00 ± 13.23
Cricket flour 15%	55.1 ± 0.15	2.92 ± 0.14	8.75 ± 0.43	41.67 ± 2.89
Factor	**Significance**			
Flour type	p < 0.001	p 0.001	–	–
Percentage of substitution	-	–	–	p 0.031
Flour type – percentage of substitution interaction	p 0.007	p 0.016	–	p 0.047

- Water absorption (WA)

Regarding WA, the ANOVA shows that the individual effect of flour type is statistically significant, while this is not the case for the percentage of substitution (Table 32). In particular, Table 32 shows differences in WA according to the different flour type (the highest for chickpea, followed by cricket and mealworm flours). A slight increasing trend in WA was highlighted in the case of cricket flour substitution, contrariwise, a decreasing trend in WA was observed with mealworm flour substitution (Table 32). This is due to the different composition of flours. As reported in Rumpold and Schlüter (2013), it could, in particular, be due to the different amino acid composition of insect protein. Concerning chickpea flour, as highlighted by Mohammed et al. (2012), increased WA appears to be due to a slight increase in total protein content and a rise in pentosans, in particular ribose and deoxyribose. This is supported by the results of the MOLS analysis, which found a statistically significant relationship between WA and protein ($p < 0.001$), fat ($p < 0.001$), and carbohydrates ($p < 0.001$) (R^2 0.89).

Furthermore, the flour type–percentage of substitution interaction was also significant (Table 32). In fact, the effect of flour type varies considerably as a function of the percentage of substitution. This may be due to their different amino acid composition and different pentosans content (Rumpold & Schlüter, 2013; Mohammed et al., 2012).

Regarding differences in WA between tested flours and the control, the results of WMWRST did not find any significant differences. These results are supported by Osimani et al. (2018) who, furthermore, found that WA increased as the percentage of cricket flour increased from 10% to 30%, consistent with the results shown in Table 32.

- Dough development time (DDT)

With regard to DDT, Table 32 shows that the effect of flour type is statistically significant, while this is not the case for the percentage of substitution. This is related to flour composition, and highlighted by the results of the MOLS analysis which found a statistically-significant relationship between DDT and protein (p 0.049), fat (p 0.047), and carbohydrates (p 0.048) (R^2 0.41). Furthermore, Mohammed et al. (2012), found an increase in DDT as the percentage of chickpea flour increased, consistent with the results reported in Table 32.

Furthermore, the WMWRST highlighted a statistically-significant increase in DDT for chickpea flours at 10% and 15%, compared to the control, which confirms the findings of Kohajdová et al. (2013). The reason for the slow DDT appears to be linked to the interaction between legume proteins and wheat proteins, which delays gluten hydration and development (Kohajdová et al., 2013; Mohammed et al., 2012). Finally, a significant result was obtained for the flour type–percentage of substitution interaction (Table 32).

- Dough stability (S)

With respect to S, the two-way ANOVA found no statistically significant differences for flour type, percentage of substitution and their interaction. Nevertheless, the WMWRST found a significant increase in S, compared to the control, for chickpea flour at 5% and cricket flour at 15%. In fact, as reported by Mohammed et al. (2012), substitution with smaller amounts of chickpea flour leads to a significant increase in S. Regarding cricket flour at 15%, the significant increase in S can be attributed to its different protein composition (Rumpold & Schlüter, 2013) and amount, as highlighted by other authors (González et al., 2019; Osimani et al., 2018).

- Degree of softening (DS)

With regard to DS, Table 32 shows that the effect of the percentage of substitution is statistically significant, while this is not observed for flour type. In fact, substitution with high amounts (i.e. 10% and 15%) of insect and chickpea flours leads to significant variation in DS (Kohajdová et al., 2013; Osimani et al., 2018). The results of the two-way ANOVA are supported by the results of the WMWRST, which found a statistically-significant reduction, compared to the control, in DS for wheat flours substituted with 10% and 15% chickpea flour, and 15% cricket flour.

As highlighted by Kohajdová et al. (2013), and Osimani et al. (2018), this is likely to be linked to the interaction between wheat protein and chickpea and insects proteins, which delays hydration and the development of the gluten network, leading to an increase in DDT and a consistent reduction in DS. This result was supported by the results of the MOLS analysis, which found a statistically significant relationship between DS and protein (p 0.008), fat (p 0.007), and carbohydrates (p 0.008) (R^2 0.38). Furthermore, the two-way ANOVA found a significant flour type–percentage of substitution interaction (Table 32).

7.2.2.3.2 Chopin Alveograph

The results of the alveograph tests and the two-way ANOVA are summarized in Table 33.

Table 33: Results of alveograph tests (mean of five measurements (diskettes) for each proof) and p-values assessed with the two-way ANOVA. Results are expressed as the mean of the three replicates ± SD. (−) indicates no significant difference at p < 0.05. (Source: Cappelli et al., 2020h).

Samples	P (Dough tenacity)	L (Dough extensibility)	G (Index of swelling)	W (Deformation energy)	P/L (Curve configuration ratio)
Control (100% wheat)	46.40 ± 1.39	57.53 ± 16.92	16.71 ± 2.41	77.13 ± 6.17	0.87 ± 0.25
Mealworm flour 5%	47.87 ± 1.68	41.87 ± 0.42	14.37 ± 0.08	66.27 ± 4.47	1.16 ± 0.03
Mealworm flour 10%	51.40 ± 2.25	30.33 ± 3.07	12.24 ± 0.66	59.40 ± 5.10	1.73 ± 0.18
Mealworm flour 15%	55.67 ± 3.72	26.47 ± 2.87	11.40 ± 0.61	57.80 ± 1.06	2.19 ± 0.36
Chickpea flour 5%	46.67 ± 2.19	65.93 ± 24.32	17.85 ± 3.19	81.40 ± 7.69	0.78 ± 0.27
Chickpea flour 10%	42.33 ± 3.01	67.53 ± 14.83	18.21 ± 2.07	72.53 ± 2.76	0.66 ± 0.21
Chickpea flour 15%	42.00 ± 2.20	46.93 ± 6.00	15.13 ± 0.93	57.80 ± 3.34	0.94 ± 0.11
Cricket flour 5%	53.07 ± 5.59	52.80 ± 12.99	16.07 ± 1.92	80.47 ± 3.75	1.08 ± 0.32
Cricket flour 10%	59.80 ± 3.67	42.80 ± 11.59	14.46 ± 1.96	80.93 ± 9.34	1.51 ± 0.48
Cricket flour 15%	67.53 ± 6.73	37.07 ± 6.77	13.50 ± 1.24	84.40 ± 2.31	1.90 ± 0.55
Factor	**Significance**				
Flour type	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001
Percentage of substitution	p 0.013	p 0.022	p 0.009	p 0.003	p 0.001
Flour type – percentage of substitution interaction	p 0.007	–	–	p 0.003	–

- Dough tenacity (P)

Regarding dough tenacity (P), the two-way anova found individual effects for flour type and percentage of substitution. As reported by Cappelli et al. (2018), in ancient wheat flours P is closely related to protein content. In fact, as reported in Table 33, P varies significantly according to the type of flour used for the substitution. In particular, it increases significantly as the percentage of cricket and mealworm flour increases, consistent with the protein rise shown in Table 31 and earlier work (Osimani et al., 2018). On the other hand, P decreases slightly in the case of substitution with chickpea flour for the dough softening, consistent with Moneim et al. (2013), and carbohydrate content remain fairly stable (Table 31). These results are supported by the MOLS analysis, which highlighted a statistically-significant relationship between P and protein (p 0.003), fat (p 0.002) and carbohydrate (p 0.002) (R^2 0.86).

The results of the WMWRST supported the results obtained with the two-way ANOVA. Notably, it found a significant increase in P compared to the control, for mealworm flours at 10% and 15%, and for cricket flours at 10% and 15%; this is due to the increase in total protein content (Cappelli et al., 2018; Osimani et al., 2018). Furthermore, the same test found a significant reduction in P compared to the control, for chickpea flour at 15%. This could be related to the presence of undesirable enzymes in chickpea flours, which interfere with gluten-forming proteins (Sabanis et al., 2006). This negative effect on dough rheology is particularly marked in the case of high percentages of substitution (Sabanis et al., 2006). In fact, the two-way ANOVA found a significant flour type–percentage of substitution interaction (Table 33).

- Dough extensibility and index of swelling (L and G)

With regard to L and G, Table 33 shows that individual effects of flour type and percentage of substitution are statistically significant, while this was not the case for their interaction. Consistent with Cappelli et al. (2018), G and L are closely related to gluten and starch content in ancient wheat flours. Our results found that both decrease as the percentage of substitution of insect flour increases (Table 33). This is due to the different composition of flours; in particular, the increase in total protein content (Table 31), which reduces starch and gluten content, with a consistent reduction in L and G. The reduction in L and G is less marked for chickpea flours, as a result of their amino acid composition, which is more similar to that of wheat (Malunga et al., 2014). Furthermore, the reduction in carbohydrate content was less marked compared to the sources of animal protein (Table 31). Finally, the WMWRST did not find any statistically-significant differences compared to the control.

➢ Deformation energy (W)

With regard to the dough strength index (W), Table 33 shows individual effects for flour type and percentage of substitution, while the flour type–percentage of substitution interaction is also statistically significant. This is due to differences in flour composition, confirmed by the results of the MOLS analysis which found a statistically-significant relationship between W and protein ($p < 0.001$), fat ($p < 0.001$), and carbohydrate ($p < 0.001$) (R^2 0.72).

Regarding chickpea flours, W decreases as the percentage of substitution increases (Table 33). This trend is consistent with the literature (Moneim et al., 2013). W increased slightly for flours substituted with 5%, 10% and 15% cricket flour (Table 33). This could be due to changes in the area under the curve of the graph. In fact, the very marked increase in P and the smaller reduction in L increases the area under the curve of the graph (increasing W). In fact, the Tukey HSD test found no significant differences between flours substituted with 5%, 10%, and 15% cricket flour respectively.

Concerning mealworm flour, Table 33 shows a decreasing trend in W as the percentage of substitution increases. Nevertheless, the Tukey HSD test found no significant differences between flours substituted with 5%, 10%, and 15% of mealworm flour. However, it is important to highlight the lack of studies of the rheological properties of wheat flours substituted with mealworm flours. The two-way ANOVA found a significant interaction between the two factors. In conclusion, the results of the WMWRST did not find any statistically-significant differences compared to the control.

➢ Curve configuration ratio (P/L)

The P/L ratio plays a key role in the technological success of leavened products. Table 33 shows that the effects of flour type and percentage of substitution are statistically significant, while this is not observed for their interaction. P/L increases as the percentage of substitution with mealworm and cricket flours increases (Table 33); this is consistent with Osimani et al. (2018). With regard to chickpea flours, the results reported in Table 33 show a decrease in P/L at 5% (which approaches the optimal value of 0.80) and 10% substitution. Conversely, the value increases in the case of 15% substitution (Table 33). The same fluctuating trend was pointed out by Moneim et al. (2013). In conclusion, the WMWRST did not highlight any statistically-significant differences compared to the control.

7.2.2.3.3 Bread characteristics

Table 34 summarizes the results of the bread characterization and the two-way ANOVA.

Table 34: Results of bread characterization and p-values assessed with the two-way ANOVA. Results are expressed as the mean of the three replicates ± SD. (−) indicates no significant difference at p < 0.05. (Source Cappelli et al., 2020h).

Samples	Bread volume (L)	Crumb density (g/ml)	Crumb moisture (%)	Crust moisture (%)
Control (100% wheat)	1.44 ± 0.04	0.28 ± 0.04	41.98 ± 0.83	24.05 ± 0.77
Mealworm flour 5%	1.31 ± 0.04	0.29 ± 0.02	41.38 ± 0.81	23.50 ± 1.01
Mealworm flour 10%	1.20 ± 0.01	0.34 ± 0.03	42.00 ± 0.17	22.74 ± 0.99
Mealworm flour 15%	1.16 ± 0.01	0.36 ± 0.02	40.71 ± 1.61	23.43 ± 0.26
Chickpea flour 5%	1.49 ± 0.01	0.27 ± 0.03	41.65 ± 1.59	24.23 ± 1.16
Chickpea flour 10%	1.36 ± 0.02	0.32 ± 0.01	41.84 ± 0.71	24.39 ± 1.48
Chickpea flour 15%	1.29 ± 0.02	0.34 ± 0.02	43.16 ± 0.97	25.51 ± 1.07
Cricket flour 5%	1.27 ± 0.02	0.32 ± 0.03	42.07 ± 0.25	22.36 ± 2.19
Cricket flour 10%	1.18 ± 0.00	0.36 ± 0.05	42.01 ± 0.36	24.96 ± 1.47
Cricket flour 15%	1.08 ± 0.02	0.38 ± 0.02	42.02 ± 0.52	23.79 ± 2.26
Factor	**Significance**			
Flour type	p < 0.001	p 0.007	–	–
Percentage of substitution	p < 0.001	p < 0.001	–	–
Flour type – percentage of substitution interaction	–	–	–	–

- Bread volume

With regard to bread volume, the two-way ANOVA found significant individual effects for flour type and percentage of substitution, while this was not the case for their interaction (Table 34). Concerning bread made with cricket and mealworm flours, bread volume decreased as the percentage of substitution increased (Table 34). This can be attributed to a reduction in dough extensibility and the weakening of the gluten network (caused by dilution, reduced hydration and interactions with non-starchy carbohydrates and non-gluten-forming proteins), which could also occur (with less pronounced effects) with chickpea flour. This, in turn, reduces the bread's gas retention capacity, as reported by Defloor et al. (1993) and González et al. (2019).

Conversely, the results reported in Table 34 show that the highest bread volume was achieved with 5% chickpea flour. However, this trend was reversed with higher substitution percentages (Table 34). The trend has been highlighted by other authors (Yousseff et al., 1976) and may be due to dough softening and reduced extensibility, which inevitably reduces gas retention capacity (Yousseff et al., 1976).

The WMWRST highlighted a statistically-significant increase in bread volume compared to the control for chickpea flour at 5%, as previously reported by Yousseff et al. (1976). This supports the results of the two-way ANOVA (Table 34). The statistical analysis also highlighted a significant decrease in bread volume with respect to the control, for mealworm flour at 10% and 15%, and for cricket flour at 10% and 15%. This might be related to the inferior rheological performances of dough substituted with insects flours (in particular on P/L values) (Table 33), and for the higher fat content of insects flours (Table 31).

- Crumb density

For crumb density, Table 34 shows that individual effects of flour type and percentage of substitution are statistically significant, while this was not observed for their interaction. With regard to bread obtained with insect flours, there is a significant increase in crumb density as the percentage of substitution increases (Table 34). These results are supported by González et al. (2019) and Osimani et al. (2018), who highlighted a significant decrease in bread specific volume for cricket flour from 10% to 30%.

Regarding bread obtained with chickpea flour, the results reported in Table 34 also show an increase in crumb density, albeit less than for insect flour, as the percentage of substitution increases. The works of Man et al. (2015) and Mohammed et al. (2012) support these findings. In particular the latter studies observed a significant reduction in bread specific volume as the percentage of chickpea flour increased. Finally, the WMWRST did not find any statistically-significant differences compared to the control.

- Crumb and crust moisture

With regard to crumb and crust moisture, the two-way ANOVA did not find any statistically-significant differences for flour type, percentage of substitution or their interaction. Furthermore, the WMWRST did not find any statistically-significant differences compared to the control. The same results were observed by González et al. (2019) in the case of substitution with insect flour. Moreover, no significant difference in crumb and crust moisture was observed by Yousseff et al. (1976) for substitution with chickpea flour ranging from 5% to 25%.

7.2.2.4 Conclusions

The results highlight that cricket and chickpea flours are suitable for the production of enriched bread. These flours could be incorporated into baked goods to improve their nutritional value, notably their protein content (Table 31) (Cappelli et al., 2020h). Nevertheless, substitution with 15% cricket flour significantly increased S and significantly reduced DS compared to the control (100% wheat flour). However, higher percentages increased P and P/L, and reduced L. This inevitably lead to a fall in volume, and other negative effects on bread characteristics. For this reason, substitution with lower percentages of cricket flour (i.e. 5–10%) seems to be the optimal choice, as it leads to satisfactory bread characteristics and nutritional improvements, as noted by Osimani et al. (2018) (Cappelli et al., 2020h).

The results of the two-way ANOVA and the WMWRST both confirm that chickpea flour at 5% was the best source of protein and, moreover, improver of wheat flour (Cappelli et al., 2020h). This substitution significantly increased S and bread volume compared to the control. Furthermore, regarding alveograph parameters, P was comparable to the control, while a slight increase in W and L, combined with a reduction in P/L (which approaches the optimal value of 0.80), were observed. The results of rheological tests were consistent with the observed increase in bread volume. Overall, the substitution with 5% chickpea flour was found to be optimal in improving rheological properties, bread characteristics and, last but not least, its nutritional value (Moneim et al., 2013; Cappelli et al., 2020h). An increased substitution of 10% and 15% chickpea flour increased DDT and decreased DS compared to the control. However, no significant improvement in dough rheology or bread characteristics was observed.

In conclusion, *Acheta domesticus* displayed better rheological and technological properties than those obtained for *Tenebrio molitor* (Cappelli et al., 2020h). This result is consistent with earlier work (González et al., 2019; Osimani et al., 2018). This might be related to the different composition of the two insect flours (Table 31). In particular to the different amino acid composition between mealworm and cricket (Rumpold & Schlüter, 2013), and for the higher fat content of mealworm flours (Table 31). However, neither dough rheology nor bread characteristics were improved by substitution with insect flour (Cappelli et al., 2020h).

On the other hand, our results show that substitution with chickpea flour, in particular at 5%, is able to improve both rheological properties and bread characteristics (Cappelli et al., 2020h). This finding could help to improve production, in particular by reducing the environmental impact. It could also help to manage problems related to annual variability, facilitating the work of agro-food chain operators (Cappelli et al., 2020h).

It seems that, sometime, in order to take a step forwards, it is necessary to take a step backwards, in this case, by rediscovering the importance of legumes as a source of high-quality food (Cappelli et al., 2020h).

8 Conclusions and future prospects

The aims of this thesis were to assess several improvement strategies for the flours production chains, with an approach from cradle to grave, and to disseminate the results of these researches to improve the two production chains that mostly needed interventions, i.e. the production chains of ancient wheats and of whole wheat flours. Moreover, the subdivision of the researches in the three main operational phases reported in section 2 (i.e. Ex-ante, Inter, and Ex-post), allow to the different stakeholders of the flours production chains, such as farmers, millers, bakers, and food technologists, to select the studies of their specific interest. The results reported in the sections 3, 4, 5, 6 and 7, clearly shows that is possible to provide technical solutions for the flours production chains which might generate significant improvement in flours, doughs, and bread.

Starting from Ex-ante (i.e. from the field), the results suggest that by means of the correct management of the agronomical treatments, such as nitrogen and sulfur fertilizations, it is possible to significantly improve the nutritional content and the technological performances of wheat kernels, flours, dough, and bread (Guerrini et al., 2020). This strategy appeared particularly effective for the technological improvement of ancient wheats flours and bread, however, it has produced excellent results also for unrefined flours like type 2 and whole wheat flours. Further studies regarding the minimum amount of fertilizers to be used and regarding other agronomic practice which could further improve the quality of wheat kernels, flours, dough, and bread are necessary; nonetheless, the results of the Ex-ante phase, provide an important starting point.

With respect to the Inter operational phase, firstly need to be examined the innovations and improvement strategies related to the wheat milling machines. This considerable part of the research highlighted that through the correct management of the milling process is possible to improve both ancient wheats and whole wheat flours. In particular, by the use of stone mill, making sure to maintain low milling temperature and stone rotational speeds, is possible to increase significantly the nutritional content of both ancient wheats and whole wheat flours (Cappelli et al., 2020i). Moreover, by the correct management of wheat conditioning (optimal moisture content 13-14% for soft wheat) is possible to improve significantly flour yield, flour quality, dough rheological properties, and bread characteristics (Cappelli et al., 2020f).

Regarding innovations and improvement strategies for roller milling, the wheat debranning before milling seems to be the most promising innovation (Cappelli et al., 2020i). This is particular interesting when bran, middlings, and germ were stabilized with new technologies such as light steam treatments, microwaves or infrared radiation (Cappelli et al., 2020i). This approach allows to stabilize and store separately the non-endospermic components of wheat (i.e. bran, middlings, and germ) that will be successively reinserted into the refined flour, thus facilitating the management of whole wheat flours orders, increasing whole wheat flour shelf life, and improving the nutritional characteristics of whole wheat bread (Cappelli et al., 2020i). Another improvement strategy for roller milling is suggested in Cappelli et al. (2020g). This strategy use the break system and the sizing and reduction systems of the roller mill for flour differentiation (Cappelli et al., 2020g). The results presented in Cappelli et al. (2020g) demonstrate that the different sections of the roller mill (i.e. break, sizing, and reduction systems) could be used to produce flours with different nutritional content and technological properties starting from the same batch of wheat, without any additional cost and without extending the duration of the milling process.

Proceeding in the Inter operational phase, this step also provide innovations and improvement strategies for dough rheology and dough kneading. In particular the results of Cappelli et al. (2018) highlighted that is possible to improve the dough rheological properties (in particular reducing tenacity (P) and tenacity/extensibility ratio (P/L), and increase dough extensibility (L)) by means of the correct management of the total water content both in refined ancient wheats flours, type 2 flours, and whole wheat flours. Moreover, to significantly improve the kneading phase, it appear to be essential to control kneading time, dough temperature, mixing speed, water absorption, water content, and dough aeration (Cappelli et al., 2020a). Despite several strategies could improve both the kneading phase (e.g. addition of organic acids, enzymes, hydrocolloids, and emulsifiers) and the kneading machines (e.g. vacuum kneading machines, automatic and adaptive kneaders), it seems that the most important parameters to be controlled during the kneading phase are the evolution of dough temperature and the correct dosage of the ingredients (Cappelli et al., 2020a).

In this direction, two specific innovations were provided by this thesis (Cappelli et al., 2020b; Cappelli et al., 2019a). The first concern the assessment of the effects related to the addition of CO_2 snow during kneading on thermoregulation, dough rheological properties, and bread characteristics (Cappelli et al., 2020b). The results reported in Cappelli et al. (2020b) clearly show that high percentages of CO_2 snow (6%, 8%, and 10%) are able to avoid dough warming during kneading and to significantly improve bread characteristics, in particular bread volume, both in ancient wheats and modern wheats flours. The second innovation proposed in the Inter operational phase was specifically developed for improving whole wheats flours, doughs, and bread (Cappelli et al., 2019a). Since that the roller mill process separates refined white flour, bran, and middlings, the idea of this innovative strategy is to delaying the addition of bran and middlings during kneading to guarantee a correct gluten development and improved whole wheat bread characteristics (Cappelli et al., 2019a). The results shows that a delay of 2 min (25% of the total kneading time) improved the dough rheology (reduced tenacity and tenacity/extensibility ratio, and increased extensibility) and whole wheat bread characteristics (greater specific volume), highlighting the interesting possibility to develop specific kneading machines for the whole wheat flours (Cappelli et al., 2019a).

Last but not least, the Ex-post operational phase. This step was focused on the increase of flours, pasta, and bakery products sustainability through the reduction of the environmental pressures by assessing the effectiveness of alternative sources of proteins and through the LCA analysis. Nowadays, the need of alternative sources of protein is becoming a critical issue (Cappelli et al., 2020e). Both the vegetable (with legumes) and the animal (with insects) worlds might provide interesting alternative sources of protein (Cappelli et al., 2020e). Regarding the effectiveness of these alternative sources of protein, in the comparison between the tested insects flours, cricket flour displayed better rheological and technological properties than those obtained for *Tenebrio molitor* (Cappelli et al., 2020h). Moreover, both insects flour produced no improvement effects on dough rheological properties or bread characteristics (just an improvement in terms of nutritional content) (Cappelli et al., 2020h). Nevertheless, only the substitution with 5% chickpea flour proved to be a significant improver for ancient wheats flours, dough, and bread. Another way to reduce the environmental impacts of the flours production chains is to assess, through LCA analysis, the impacts related to each single process of the supply chain (i.e. wheat cultivation, raw material processing and production, packaging, final product transportation, and finally, the waste, packaging and pallet disposal). As shown in Recchia et al. (2019), through the separate evaluation of the environmental impacts related to each process of the production chain, it is possible to provide specific improvement strategies that can lead to a significant reduction of the environmental pressures.

Regarding the future prospects of the research, this thesis provides several innovations and improvement strategies which, if are immediately adopted, could lead to significant ameliorations for the production chains of ancient wheats and whole wheat flours. Nonetheless, further investigations need to be made to improve the essentials unitary operations investigated in the Ex-ante, Inter, and Ex-post operational phases, i.e. wheat cultivation, wheat milling, dough kneading, and finally, the assessment of the environmental impacts related to the entire production chain.

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Appendix A. Scientific dissemination

Books

1) Cappelli, A. (2020). L'arte della macinazione: Tecniche, effetti sui prodotti e strategie di miglioramento. *Chiriotti Editori,* ISBN 978-88-96027-51-6 *(in press).*

Papers in international indexed journals

1) Cappelli, A., Bettaccini, L., Cini, E. (2020a). The kneading process: A systematic review of the effects on dough rheology and bread characteristics, including improvement strategies. *Trends in Food Science and Technology,* 104, 91–101. Doi: https://doi.org/10.1016/j.tifs.2020.08.008.

2) Cappelli, A., Canessa, J., Cini, E. (2020b). Effects of CO_2 snow addition during kneading on thermoregulation, dough rheological properties, and bread characteristics: a focus on ancient and modern wheat cultivars. *International Journal of Refrigeration,* 117, 52–60. Doi: https://doi.org/10.1016/j.ijrefrig.2020.04.006.

3) Cappelli, A., Cini, E. (2020c). Will the COVID-19 pandemic make us reconsider the relevance of short food supply chains and local productions?. *Trends in Food Science & Technology*, 99, 566. Doi: https://doi.org/10.1016/j.tifs.2020.03.041.

4) Cappelli, A., Cini, E., Guerrini, L., Masella, P., Angeloni, G., & Parenti, A. (2018). Predictive models of the rheological properties and optimal water content in doughs: An application to ancient grain flours with different degrees of refining. *Journal of Cereal Science*, 83, 229–235. Doi: https://doi.org/10.1016/j.jcs.2018.09.006.

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1) 08/04/2019 – 09/04/2019. Oral presentation at 2nd International conference on nutrition, food science and technology, Abu Dhabi (EAU).
   Title: Strategies to improve the performances of bakery products made from ancient wheats.
   Abstract:
   https://www.longdom.org/conference-abstracts-files/strategies-to-improve-the-performances-of-bakery-products-made-from-ancient-wheat8217s.pdf

Conference

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