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UNREFINED WHEAT FLOURS IN BREAD-MAKING: TECHNIQUES AND TECHNOLOGIES TO IMPROVE DOUGH PROPERTIES AND BREAD QUALITY

Settore scientifico disciplinare AGR/15

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1. Abbreviations

°C	Celsius degrees
^{1}H NMR	Proton Nuclear Magnetic Resonance
AH_2	Ascorbic acid
ARF	Aleurone-rich flour
AWFs	Ancient wheat flours
AWs	Ancient wheats
AWVs	Ancient wheat varieties
AXF	Arabinoxylan-enriched flour
B	Micronized and air-classified thin fraction of bran
BU	Brabender Units
CBP	Chorleywood Bread Process
CFP	Carbon Footprint
cm	Centimeters
CMC	Carboxymethylcellulose
CPMG	Carr-Purcell-Meiboom-Gill
CSB	Chinese steamed bread
cv	Cultivar
d	Day
DATEM	Diacetyl tartaric esters of monoglycerides
DB	Debranning
DDT	Dough development time
DOE	Design Of Experiment
dm	dry matter
DS	Dough stability
DW	Dough weakening
EVOO	Extra virgin olive oil
FID	Free induction decay
FN	Falling Number
FT	Fourier transform
G	The swelling index
g	Gram
G’	Storage modulus
G’’	Loss modulus
GBF	Gelatinized brown flour
GF	Gelatinized flour
GG	Guar gum
GHG	Greenhouse gases
GLCM	Grey-level co-occurrence matrix
GTP	Green tea powder
HPMC	Hydroxypropyl methylcellulose
hr	Hours
Hz	Hertz
J	Joule

kg	Kilograms
kHz	KiloHertz
L	The dough extensibility
L	Litre
LAB	Lactic acid bacteria
MC	Methylcellulose
mg	Micrograms
MHz	Megahertz
min	Minutes
MIR	Mid infrared spectroscopy
mL	Millilitre
mm	Millimeter
MP	Megapixel
ms	Milliseconds
N	Newton
NaCl	Sodium chloride
NIR	Near infrared spectroscopy
nm	Nanometers
P	The dough tenacity
P	Kneader power
p-value	Threshold value of the statistical probability of obtaining test results
P/L	The ratio of dough tenacity to extensibility
PCA	Principal component analysis
PG	Psyllium gum
popA	Population A
popB	Population B
popC	Population C
popD	Population D
popE	Population E
popF	Population F
ppm	Parts per million
PUFA	Polyunsaturated fatty acids
RM	Relative moisture
rpm	Rounds per minute
s	Seconds
SDBA	Second derivative band areas
sp	Specie
SSL	Sodium stearoyl lactylate
Suc	Sucrose
T	Torque
T_{2C}	Relaxation time of population C
T_{2D}	Relaxation time of population D
T_{2E}	Relaxation time of population E

T_{2F}	Relaxation time of population F
T_A	Relaxation time of population A
T_B	Relaxation time of population B
TG	Tara gum
TPA	Texture profile analysis
US	Ultrasounds
UWF	Unrefined wheat flour
W	The flour strength
w	Weight
W	Watt
WA	Water absorption
WB	Wheat bran
WG	Wheat germ
WI	Work input
WWF	Wholewheat flour
XG	Xanthan gum
μm	Micrometers
μs	Microseconds
ω	Angular velocity

2. *Introduction*

2.1 Summary

In recent years the concept of food quality has markedly changed. From the Industrial Revolution to the 90’s the technological quality became the main criterion driving millers and bakers’ choices. However, recently consumers are increasingly interested in foods with high nutritional value and low environmental impact. As a consequence, the demand for healthy and functional foods has grown greatly leading to a higher interest for the exploitation of unrefined wheat flours in the bread-making process (Schaffer-Lequart et al., 2017). Indeed, many scientific evidences reported that the regular consumption of unrefined cereal products has positive effects on human health (Hauner et al., 2012; Ye et al., 2012). However, the use of unrefined wheat flours in the bread-making process poses many technological issues since the germ and bran negatively affect dough properties and bread quality (Hemdane et al., 2016; Boukid et al., 2018). The different chemical compostion of unrefined wheat flours may require changes in the processing conditions which should be specifically designed to improve the bread-making performance of these raw materials. Bread is the staple food in many diets, hence the possibility of increasing the nutritional value of the product while maintaining an acceptable technological quality could significantly impact human health.

At first, we decided to make an in depth analysis of the literature about “Techniques and technologies for the breadmaking process with unrefined wheat flour” (article 1) in order to obtain a scientific state of the art. The main results that the review of the literature revealed were the following (i) unrefined wheat flours are processed using the standard bread-making process and evaluated adopting the quality tests developed for refined wheat flours; (ii) the main strategy the scientific research has developed to improve and standardize the performance of unrefined wheat flours is the addition of bread improvers, while the bread-making process remained almost unchanged (Parenti et al., 2020a).

The above literature overview allowed us to identify the aim of the thesis: the development of bread-making techniques and technologies specifically designed to improve the quality of dough and bread from unrefined wheat flours. A different approach for the bread-making is required since the desired features for the end product have changed from the technological quality to both nutritional and technological quality and the characteristics of unrefined wheat flours are different compared to refined flours. The standardization of raw materials and processing conditions are not appropriate to exploit the high nutritional value of unrefined wheat flours while obtaining high quality products, but different approaches are required. Although in the literature few research has been made on technologies to improve the bread-making performance of unrefined wheat flours, unrefined breads are available in the current market. Bakers have probably been the first to face the issue of using unrefined flours for bread production; hence we decided to perform a survey "The bread making process of ancient wheat: A semi-structured interview to bakers" (article 2) in order to obtain the current empirical state of the art. The empirical techniques and technologies performed in the baking industry were the opposite compared to those developed in the scientific literature: bakers do not use improvers but they have developed processing strategies for unrefined wheat flours. This means that consumers are attracted by clean label formulas, and that scientific research has not properly addressed the market requirements (Guerrini et al., 2019).

The Doctoral research focused at the beginning on improving the bread formula of unrefined wheat flours. The first study investigated the effect of a natural bread improver made with the same basic ingredients of bread dough but in a different physical form which resulted in the article 3 "The effect of the addition of gelatinized flour on dough rheology and quality of bread made from brown wheat flour". Positive results were obtained on dough properties and bread quality (Parenti et al., 2019). Since the use of improvers seems to be the most popular choice in literature, the use of different combinations of several improvers was tested. The article 4 "Breadmaking with an old wholewheat flour: Optimization of ingredients to improve bread quality" proposed a DOE-based approach for the optimization of unrefined bread formula. The article focuses on the following aspects: (i) the simultaneous effect of common and natural bread improvers on the performance of an unrefined wheat flour; (ii) the optimization of the bread formula adapting the recipe to the specific flour requirements (Parenti et al., 2020b).

Then, the thesis research moved on investigating innovative strategies for the bread-making process of unrefined wheat flours. We realized that the kneading operation, commonly considered as one of the most important step of the bread-making process, is probably the most critical operation for unrefined wheat flours. Unrefined doughs are often characterised by short kneading time, poor stability at kneading, high degree of softness resulting in sticky doughs with high tenacity and low extensibility and flour strength. Hence, we tried to better understand the physical-chemical phenomena occurring during the kneading operation of an unrefined wheat dough. Particularly, we wanted to gain a deeper knowledge on the changes in proton mobility and dynamics during kneading when an unrefined flour was used. In article 5 "Use of the ^{1}H NMR technique to describe the kneading step of wholewheat dough: the effect of kneading time and total water content", the ^{1}H NMR technique was applied for the first time to monitor the dough development. Two different dough water contents were also investigated. The aim of the study was to gain a molecular insight of the physical-chemical status of flour biopolymers and water redistribution between flour constituents in function of the kneading time and water content. Results were interpreted as physical-chemical trasformations occurring to the main flour components during kneading: the starch and gluten hydration, the glass-rubbery transition of amourphous starch regions and amourphous proteins, and the gluten network development (Parenti et al., 2021).

These studies showed that the introduction of innovative processing techniques, specifically adapted to the inherent characteristics of the raw material could improve the bread-making performance of unrefined wheat flours, promoting the consumption of healthy breads. The better comprehension of the phenomena occurring to the constituents of unrefined wheat flour during the process could increase the scant scientific knowledge on this topic as well as suggest processing strategies to improve dough properties and bread quality.

Future developments of the Doctoral thesis include the investigation of a common practice performed by bakers using unrefined wheat flour for bread-making: the gradual addition of basic bread ingredients (i.e., flour or water) during the kneading operation on dough molecular and rheological properties and bread quality (Parenti et al., submitted to *Journal of Food Engineering*).

Considering the crucial role that the kneading operation plays on the bread-making performance of unrefined wheat flours, and considering the poor mixing stability of such flours, the thesis research will also focus on the improvements of measurements methods to determine the optimal dough development, i.e. the dough readiness. An in-depth analysis of the literature about all the available methods to determine the dough readiness was performed and results highlighted the necessity to improve the determination of the optimal dough development (Parenti et al., submitted to *Journal of Food Engineering*).

The introduction of processing techniques specifically designed for the inherent characteristics of unrefined wheat flours and the possibility to improve the determination of the optimal dough development could result in better dough properties and bread quality, hence promoting the consumption of healthy breads.

A conceptual map of the Doctoral thesis research process is reported in Figure 1.

After this first Summary of the thesis, the main sections included are the following: (ii) Introduction, (iii) Aim of the thesis, (iv) Results and Discussion, (v) Conclusions and Future developments and (vi) References. In the Introduction and Results and Discussion sections, paragraphs titled according to the main topic addressed are included. Each of these paragraphs include two sub-paragraphs:

- A preliminary-remark, which connects the scientific articles reported giving an overview of the thesis research process;
- The scientific article resulting from the thesis research study.

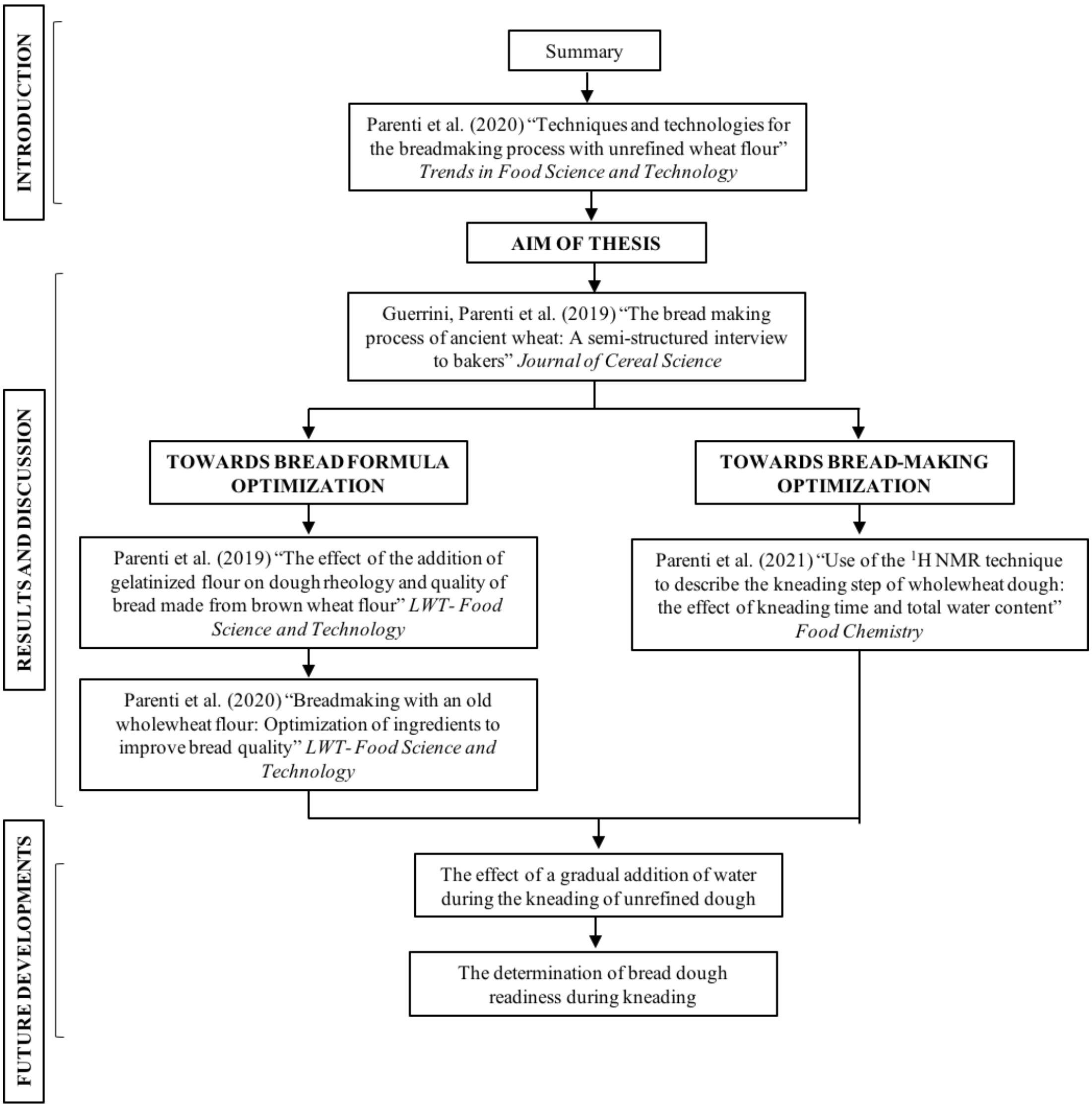

Figure 1 Schematic representation of the thesis research study.

2.2Literature state of the art

2.2.1 Preliminary remark – article 1

In recent years the concept of food quality for consumers has greatly changed compared to the previous century. Concerning cereal products, which have been at the basis of human nutrition since antiquity, a deep change was brought by the Industrial Revolution with the introduction of a new milling method, the roller milling. Although the practice of milling and sifting flours has a long history, before Industrial Revolution only single-stream flours could be obtained. This means that the original parts of the wheat kernels (i.e., bran, germ and endosperm) stay together from the beginning to the end of the milling process. Refined flours could be obtained by passing single-stream flours through sifters, a practice that only partially removed the bran and germ fractions. The roller milling method represented the first milling process that allowed to separate the starchy endosperm from the bran and germ fractions at the beginning of the process, leading to a greater yield in refined flour and better separation of the milling by-products. The resultant refined flours showed a peculiar chemical composition mainly composed by the starch and the storage proteins which results in a better technological performance, more appreciated sensory attributes (i.e. taste, flavour, and colour), higher food safety, and longer shelf-life compared to unrefined flours. The above technological innovation triggered a marked change on the wheat production-chain: refined flours could be stored for longer periods and the necessity to have mills in each town disappeared, leading to the advent of the large-scale distribution of flours with low-cost and longer shelf-life. Owing to its superior technological features, and its sudden large availability and low-cost, the use of refined flours almost completely replaced that of unrefined flours in the production of wheat based products (Zhou et al., 2014; Cauvain et al., 2015; Jones et al., 2015).

Besides the processing innovations brought by the Industrial Revolution, between the 1930s and the late 1960s, the third Agricultural Revolution occurred (i.e. the Green Revolition), which involved research and technological transfer initiatives that led to the selection of modern high-yelding wheat varieties with a superior technological quality (Dinu et al., 2018; Mefleh et al., 2019).

The driving force for all these technological and agronomical innovations was mainly represented by the technological quality of wheat flours. This means that other aspects contributing to food quality, such as the nutritional implications that this sudden change may produce on human beings as well as the environmental impacts, were not considered.

In this scenario, standard processing methods for the bread-making process were developed, which were specifically designed to optimize the performance of refined flours with good technological quality. Since wheat bread is one of the most ancient and widespread foods of all over the world, there has been a high differentiation of the product over time and space. Hence, different bread-making processes have been developed and standardized as a function of the specific characteristics the final product should present. Along with the standardization of the bread-making process, techniques to standardize the flour characteristics were developed. Indeed, despite the introduction of high-yielding wheat varieties and the use of refined flours, the flour technological quality can markedly change as a function of several variables such as wheat genotype, growing season, agronomic practices, the milling operating conditions etc. Therefore, in the baking industry, a common practice to standardize the technological quality of a wheat flour was (and is still) the use of different wheat cultivars blended together in order to obtain a final product with standard quality parameters, i.e. chemical (ash and protein contents) and rheological (Farinograph and Alveograph) parameters, according to flour destination use. This means that the raw material had to present standard technological characteristics to be successfully processed in bread with high technological quality using standard bread-making methods (Zhou et al., 2014; Cauvain et al., 2015; Jones et al., 2015).

Overall, these technological changes showed that in the past decades, consumers and baking industries gave a superior importance to the technological quality of wheat flours, which was considered a more relevant feature compared to the other factors that contribute to the complex concept of food quality.

In recent years, several scientific articles have shown that a regular consumption of unrefined cereal products has potential health benefits such as the prevention of chronic-degenerative diseases (cardiovascular diseases, type 2 diabetes, certain typologies of cancer) and improved body weight regulation (Hauner et al., 2012; Ye et al., 2012).

It is well-known that unrefined wheat flours have a different chemical composition compared to refined wheat flours. According to their refinement degree, unrefined wheat flours include different amounts of the outer layers of the wheat kernel, i.e. the bran and the germ fractions, which are rich in dietary fibre, minerals, vitamins and oils. The presence of the bran and the germ fractions positively affects the nutritional quality of the flour, enhancing its nutritional value. Unfortunately, the outer layers of the wheat kernel negatively impact the dough properties and quality of bread. Unrefined flours, although being healthier than refined flours, have weaker technological properties, resulting in sticky doughs with high tenacity, low strength and low extensibility. Unrefined breads show poorer technological quality than refined breads: lower volume, denser and grainier crumb, higher crumb firmness, darker colour and worse sensorial characteristics, which are mainly related to the bitter taste caused by the presence of wheat phenolic compounds. Furthermore, the shelf-life of unrefined wheat flours is much shorter than refined flours; the richer chemical composition of unrefined flours is susceptible to several deterioration phenomena such as the fast development of the rancidity (Boukid et al., 2018; Hemdane et al., 2016; Heinio et al., 2016).

At present time, the consumers' demand for healthy foods has grown greatly. Indeed, the nutritional quality has become a central quality criterion driving food choices, probably due to the the scientific evidences showing the positive health benefits of a regular intake of unrefined cereals (Hauner et al., 2012; Ye et al., 2012). However, the consumption of unrefined wheat products is still much lower to reach the recommended daily fibre intake due to both technological issues and consumer sensory preferences (Heinio et al., 2016; Stephen et al., 2017). As a consequence, the scientific research and the food industry have been focusing on the development of strategies and technologies to produce healthy and functional foods.

We considered this topic very interesting, and characterised by implications in different areas, ranging from the scientific research, to the baking industry, the environmental sustainability and the human health.

The first study of the present Doctoral thesis was a review article titled "Techniques and technologies for the breadmaking process with unrefined wheat flours". The article includes an in-depth analysis of the scientific literature about the current techniques and technologies that have been developed to improve the technological performances of unrefined wheat flours and represents the basis for the identification of the scope of the Doctoral research study.

2.2.2 Article 1

Parenti, O., Guerrini, L., & Zanoni, B. (2020). Techniques and technologies for the breadmaking process with unrefined wheat flours. *Trends in Food Science & Technology*, 99, 152–166. https://doi.org/10.1016/j.tifs.2020.02.034

Techniques and technologies for the breadmaking process with unrefined wheat flours

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Abstract: Background: In recent years there has been an increasing interest in the production of wholegrain products owing to the positive effects shown on human health. Although refined flour still represents the standard reference in breadmaking technology, consumer demand for unrefined breads has grown greatly. The different chemical composition of unrefined wheat flours (UWFs), which includes specific fractions of milling by-products (i.e., wheat bran and wheat germ), favours the nutritional value, but it has a negative effect on technological performance. Therefore, it is useful to develop new strategies specifically designed to improve the quality of UWF breads.

Scope and approach: The present review aims to set out the techniques and technologies that have been reported in the literature for the breadmaking process with UWFs, that is, from raw material processing to bread formulation and breadmaking methods.

Key findings and conclusion: The evaluation of UWF quality is still based on the tests developed for refined flour, which cannot properly estimate UWF technological properties. The greatest efforts to improve the breadmaking performance of UWF have been focused on modifying the bread formula, mainly with the addition of improvers. Conversely, very little investigation has been carried out on adapting the breadmaking process to the different characteristics of the raw material. Overall, the use of UWF in breadmaking may require further investigations into processing strategies to improve the quality of the end product, hence increasing the consumption of healthy foods.

Keywords
bread; wholewheat flour; milling by-products; wheat germ; wheat bran

Abbreviations
unrefined wheat flour (UWF), wheat germ (WG), wheat bran (WB)

Trends in Food Science and Technologies (2020) 99, 152-166

1. Introduction

Wheat bread is the staple food in many diets, representing one of the main sources of the daily energy intake. Bread composition is the result of several factors including wheat genotypes, agronomic treatments, environmental conditions, flour composition, breadmaking conditions and product storage.

The wheat species most widely used for breadmaking is Triticum aestivum L. or "common" wheat, accounting for 95% of wheat production, followed by Triticum durum or "durum" wheat, which is widely used in Mediterranean cuisine for making special breads, couscous, pasta and bulgur.

For most of human history, flour was produced using stone mills, which simultaneously crushed and ground wheat kernels in a single millstream, giving wholewheat flour. The milling process was completely revolutionized during the second half of the 19th century with the introduction of the roller mill, which allowed the three fractions of the caryopsis (i.e., starchy endosperm, bran and germ) to be separated at the beginning of the process, resulting in different millstreams (Jones, Adams, Harriman, Miller, & Van der Kamp, 2015). The refined flour obtained from the starchy endosperm alone shows better technological quality and gives breads with sensory properties that are widely appreciated by consumers. Hence, refined flour has become the standard for the further technological developments in the bakery industry up to the present day. This means that all the knowledge on bread formulation and process implementation has been made by considering refined wheat flour, characterized by a chemical composition mainly composed of starch (80%-85%) and proteins (8%-14%). The other millstreams (i.e., bran, germ) are instead considered milling by-products and mainly used as animal feed.

However, in recent years there has been renewed interest in unrefined wheats, since several studies have shown that regular consumption of these products is associated with health benefits such as a lower risk of chronic-degenerative diseases and improved body weight regulation (Ye, Chacko, Chou, Kugizaki, & Liu, 2012; Hauner et al., 2012) The increased interest in healthy and functional foods has led to a consequent growth in the demand for high nutritional value breads (Gani, & Hameed, 2012). As a result, it has been necessary to re-interpret bread quality, also including nutritional value.

Unrefined wheat flour (UWF) is a composite class which includes flours supplemented with milling by-products at the same or a different relative proportion compared to that of the intact caryopsis (Fig. 1). The official definition for flours containing the same components in the same relative proportions as the wheat kernel is "wholewheat" flour

(Whole Grain Initiative approved by ICC, Healthgrain and Cereal & Grain Association, 2019), although the modern milling process does not allow for the inclusion of wheat germ (WG), but only recovers wheat bran (WB). Hence, the resulting "wholewheat" flour is no more than flour enriched with bran.

Despite the nutritional benefits, the introduction of milling by-products in the breadmaking process has some drawbacks, the solutions to which are still open challenges for bread makers.

First, the outer layers are the most susceptible to contaminants, i.e., mycotoxins and heavy metals (Sovrani et al., 2012). Hence, the possibility of using milling by-products to produce high nutritional value breads requires an integrative approach from the field to the breadmaking. Therefore, appropriate agronomical and/or post-harvest strategies must be adopted to reduce the safety risk and preserve the high nutritional value of the outer layers. Secondly, WG and WB negatively affect the technological quality of doughs and breads (Boukid, Folloni, Ranieri, & Vittadini, 2018; Hemdane et al., 2016). Finally, since the sensorial characteristics of unrefined bakery products are little appreciated by consumers, several scientific studies have dealt with the sensorial profile to increase their acceptability (Gani at al., 2012; Heiniö et al., 2016).

The present review aims to report the processing strategies that have been developed until now for the breadmaking process with UWFs (Fig.2), while suggesting some processing innovations to improve the exploitation of UWFs.

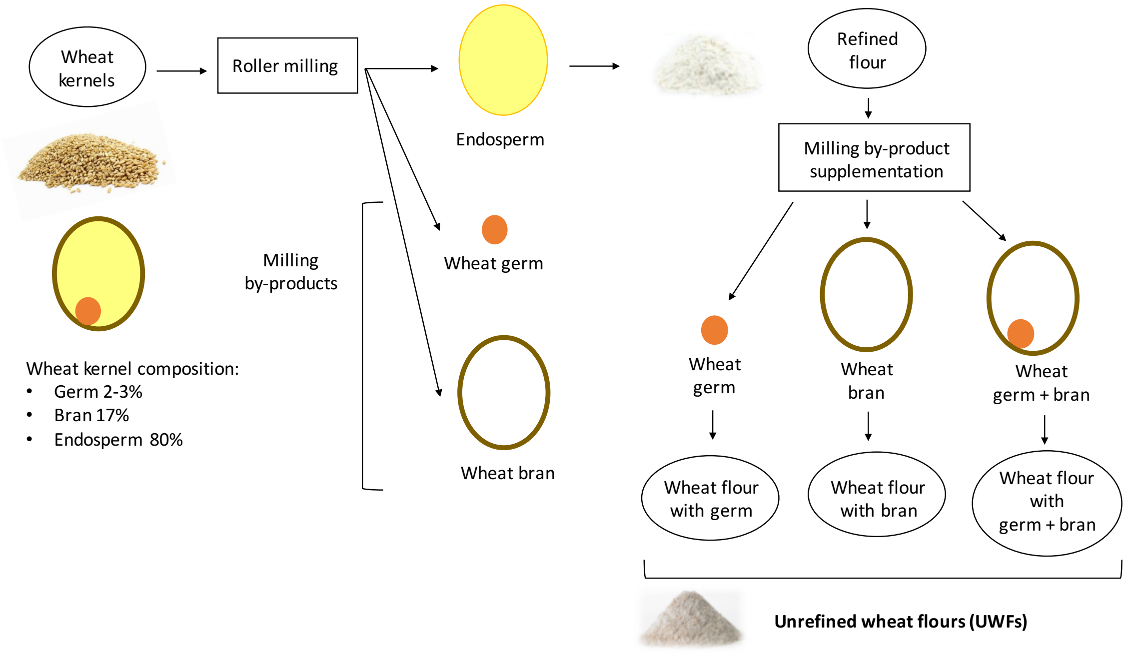

Fig. 1 Schematic representation of the production of unrefined wheat flours (UWFs): flour enriched with wheat germ, flour enriched with wheat bran, and flour enriched with both wheat germ and bran in the same (i.e., wholewheat flour) or in a different relative proportion to the wheat kernel.

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2. Bread quality

According to the common good manufacturing practices, "bread" must be prepared by baking a dough which consists of flour, yeast, and a moistening ingredient, usually water. In the present review this term refers to all typologies of leavened breads, not including flat breads obtained without any leavening. Bread is one of the most ancient and widespread foods of all over the world. Progressive technical developments over many thousands of years have led to a high diversification of the product. Hence, a "good bread" presents different features depending on cultural background, individual experiences, and personal likes and dislikes. Moreover, quality features change over space, in different regions, and over time, together with food technology innovations.

However, despite the large diversity of bread characteristics, in the literature bread quality is mainly evaluated as: (i) bread specific volume, (ii) crumb characteristics and (iii) crust colour (Zhou et al., 2014; Cauvain, 2015). These features are the results of the raw materials and processing conditions adopted during bread production. Almost all of the quality characteristics are related to the gluten network, since it traps the gas produced during the leavening and contributes to the formation of a cellular crumb structure which confers the bread's volume, texture and eating qualities. The addition of the "right" quantity of water is another key factor affecting dough rheology and the development of gluten; too much or too little water means that the right gluten network cannot be properly developed (Cauvain, 2015).

Although there are not any standard sensorial attributes of wheat bread, they are all considered of maximum importance in the evaluation of the product. Sensorial features affect consumers' preferences and drive their choices towards the different bread typologies proposed by the current bakery market.

The flour used in the bread formulation plays a critical role for the product characteristics. Refined bread presents a high loaf volume, a light colour, homogeneous crumb porosity and soft crumb. Refined flour results in a fairly bland sensorial attribute with only a slight grain-like flavour or malted note, since most of these sensory contributions come from the WG oils and from the WB particles removed during milling (Callejo et al., 2015; Challacombe, Abdel-Aal, Seetharaman, & Duizer, 2012; Eckardt et al., 2013; Hayakawa, Ukai, Nishida, Kazami, & Kohyama, 2010; Heenan, Dufour, Hamid, Harvey, & Delahunty, 2008; Jensen, Oestdal, Skibsted, Larsen, & Thybo, 2011a; Katina, Heiniö, Autio, & Poutanen, 2006a; Lotong, Edgar-Chambers, & Chambers, 2000). The sensory attributes of refined breads are largely appreciated by consumers, who, despite the proven health benefits of wholewheat consumption, still prefer refined products (Ye, Chacko, Chou, Kugizaki, & Liu, 2012).

The distinctive characteristics of UWF breads include a low loaf volume, coarse and hard texture, dark colour and "speckled" appearance. Moreover, they are characterized by a nutty odour, bitter/sour taste, a grain-like, "seedy" flavour, malted note and musty attribute (Curti, Carini, Bonacini, Tribuzio, & Vittadini, 2013; Callejo et al., 2015; Challacombe, Abdel-Aal, Seetharaman, & Duizer, 2012; Eckardt et al., 2013; Heenan, Dufour, Hamid, Harvey, & Delahunty, 2008; Jensen, Oestdal, Skibsted, Larsen, & Thybo, 2011a; Katina, Heiniö, Autio, & Poutanen, 2006a; Katina, Salmenkallio-Marttila, Partanen, Forssell, & Autio, 2006b).

The sensory profile of UWF breads is one of the major obstacles to increasing their consumption. The most challenging attribute is probably the bitter taste, associated with the presence of bioactive compounds (such as phenolic compounds, amino acids, small peptides, fatty acids and sugar) which are highly concentrated in the outer layers of the wheat kernel (Heiniö, 2009; Zhou et al., 2014; Van Gemert, 2011; Mattila, Pihlava, & Hellström, 2005); rancid sensory defects could also occur relating to oxidation of the WG oil (Heiniö et al., 2016).

The quality characteristics were created for refined breads, but in the literature they are also used for UWF bread. However, well-informed consumers appreciate the better nutritional value of UWF and they have different expectations about the product characteristics.

This can lead implicitly to some questions on bread quality. Does the different chemical composition of UWF require new qualitative standards or are the quality criteria of refined flours still suitable for UWF? Is it right to have the same expectations about the characteristics of the final product? It is hard to answer these questions and they require an in-depth discussion from a wide perspective.

Besides bread quality criteria, some important methods have also been developed for refined flours. This is the case of the optimum water absorption (WA) of the flour, officially determined as the amount of water required to reach 500BU in the farinographic test. However, it has been reported that 500BU underestimates the absorption capacity of UWF (Hemdane et al., 2016). Improper hydration of the dough could lead to i) the wrong evaluation of UWF bread quality, especially when compared to refined bread, and ii) biased results if a new process setting is desired and the tested variables significantly interact with water. Nevertheless, indications regarding the correct amount of water for UWFs are still missing in the literature.

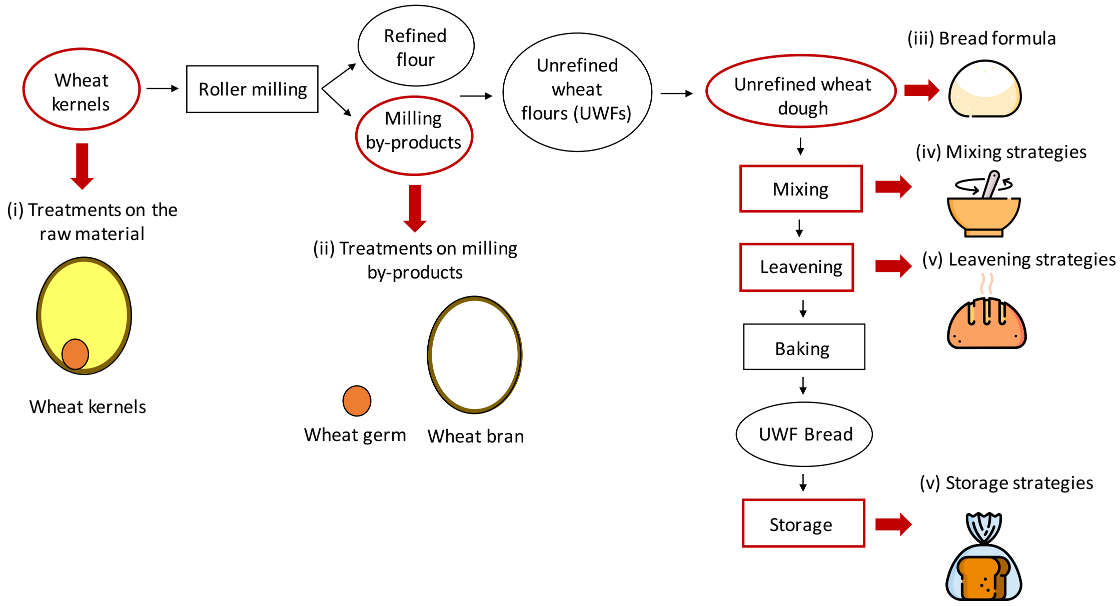

Fig. 2 Schematic representation of the main techniques and technologies reported in the literature for the breadmaking process with unrefined wheat flours (UWFs). (i) Treatments on the raw material (i.e., wheat kernels) before the milling step; (ii) treatments on milling by-products (i.e., wheat germ and bran); (iii) modification of the bread formulation; (iv) modifications of processing variables: mixing, leavening and (v) improvement of bread storage.

3. Breadmaking process

The literature has shown controversial results regarding breadmaking with UWFs, making it difficult to interpret or compare experimental data in the literature. Indeed, several operating factors have been reported to have a significant effect on bread quality (Table 1):

(i) The composition of UWFs is extremely variable; beside the starchy endosperm they include the presence of specific fractions of milling by-products, WG and WB. The different chemical compositions of UWFs produce great variability in the breadmaking performance.

(ii) The milling method. It is widely known that the milling method has a marked impact on the technological performance of flour (Jones, Adams, Harriman, Miller, & Van der Kamp, 2015). Standard indications about the milling method, and particle size and composition of milling by-products could be useful for a better comprehension of their impacts and could help overcome their adverse effects in breadmaking. It is important to point out that the milling method strongly affects the UWF composition.

(iii) Bread formulation. In the literature there is no official bread formula, but different recipes are adopted. Besides the basic components (i.e., flour, water and the leavening agent), other ingredients are often added to the bread dough, such as salt (NaCl), sugar, shortening and oxidizing agents, which affect the flour performance. This variability of recipes could hinder the comparison and understanding of the effects and results.

(iv) Breadmaking procedure. Owing to the absence of a standard process in breadmaking, operating conditions cannot be standardized, making it difficult to compare and understand the literature data.

The paragraphs below set out to improve the comparability between the different studies in the literature by promoting both a standardization of methods and some technological innovations for UWF processing.

Food matrix/ Process step	Processing strategy
Wheat kernels	Germination
Milling by-products	Bran pre-soaking Bran/germ fermentation
Bread dough	Bread formulation -Optimization of water amount -Addition of modified flour • Pre-gelatinized flour • Waxy wholewheat flour -Addition of improvers • Enzymes (xylanase, alfa-amylase, G4 amylase) • Hydrocolloids (carboxymethylcellulose CMC, guar gum GG, hydroxypropyl methylcellulose HPMC, methylcellulose MC, psyllium gum PG, xanthan gum XG, tara gum TG) • Oxidants (ascorbic acid, rosehip, potassium bromate) • Emulsifiers (diacetyl tartaric esters of monoglycerides DATEM, sodium stearoyl lactylate SSL, ethoxylated monoglycerides, succinylated monoglycerides, lecithin, polyoxyethylene sorbitan monostearate, polyoxyethylene sorbitan monopalmitate, glycerol-monostearate) • Vital gluten
Mixing	Mixing time Delayed addition of milling by-products
Leavening	Sourdough fermentation
Bread storage	Treatments on milling by-products (fermentation) Bread formulation -Addition of modified ingredients • Pre-gelatinized flour -Addition of improvers • Enzymes (xylanase, alfa-amylase, G4 amylase) • Emulsifiers (diacetyl tartaric esters of monoglycerides DATEM, sodium stearoyl lactylate SSL, monoglycerides) • Hydrocolloids (carboxymethylcellulose CMC, guar gum GG, hydroxypropyl methylcellulose HPMC, dextran) • Malted flour • Anti-oxidants (alfa-tocopherol, rosemary extract, green tea powder, microencapsulated n-3 polyunsaturated fatty acids PUFA powder)

Table 1. The main processing strategies for breadmaking with unrefined wheat flours (UWFs).

3.1 UWF quality and composition

In recent years several studies have reported the nutritional value, technological impact and sensory profile of the milling by-products WG and WB in the breadmaking process (Hemdane et al., 2016; Boukid, Folloni, Ranieri, & Vittadini, 2018; Heiniö et al., 2016). WG (2%-3% of the caryopsis) is composed of the embryo and scutellum, the latter being discarded with the bran during the milling process (Boukid, Folloni, Ranieri, & Vittadini, 2018). It is considered the most nutritious part of the wheat kernel, providing 381 cal/100 g: 54% carbohydrates, 23% proteins and 23% lipids (Boukid, Folloni, Ranieri, & Vittadini, 2018; Nutrition data, 2014).

The limited utilization of WG in the bakery industry is primarily due to the high presence of unsaturated fats and hydrolytic and oxidative enzymes (i.e., lipoxygenase and lipase) which favour WG degradation (Boukid, Folloni, Ranieri, & Vittadini, 2018). Several treatments have been developed to improve WG stability while preserving the high nutritional value (Boukid, Folloni, Ranieri, & Vittadini, 2018). The first crucial step is to perform an efficient separation of the WG from the other flour components. Two different approaches have been developed: (i) direct degermination, which is performed before the milling process and (ii) indirect degermination, which is realized through gradual separation phases during the milling process (Boukid, Folloni, Ranieri, & Vittadini, 2018). A high recovery of the WG fraction enables the subsequent adoption of stabilization strategies through the deactivation of the oxidative enzymes or/and removal of the oil fraction. These strategies are based on physical, chemical or biological methods, as extensively revised by Boukid, Folloni, Ranieri, & Vittadini (2018).

WB (17% of the caryopsis), together with the aleurone layer and remnants of the starchy endosperm and WG, produces a range of milling by-products which are recovered at different stages in the mill (Hemdane et al., 2016). The bran fractions can be classified as different by-products (i.e., coarse bran, coarse weatings, fine weatings and low-grade flour), which are roughly distinguishable based on two main characteristics: particle size and endosperm content (Hemdane et al., 2016).

The bran streams recovered further down the milling process consist of finer bran particles and contain relatively more endosperm (Hemdane et al., 2016). Coarse bran mainly consists of non-starch carbohydrates, with 17%-33% arabinoxylan (AX), 9%-14% cellulose, 3%-4% fructan and 1%-3% mixed-linkage β-D-glucan as major components (Hemdane et al., 2016). In order to study the mechanisms through which bran affects breadmaking, multiple approaches which change bran functionality (physical properties, chemical composition, and/or enzymatic load) have been developed. These strategies (i.e., (i) particle size reduction, (ii) (hydro-)thermal treatments, (iii) pre-soaking, (iv) enzymatic treatment, (v) fermentation, and (vi) chemical treatment, reviewed by Hemdane et al., 2016), treat the bran before its re-addition to the flour, and investigate both bran properties and breadmaking performance.

In the literature, great efforts have been made to try to understand the functionality of milling by-products or to develop new strategies to stabilize them. Conversely, far fewer efforts have been made to try to improve the breadmaking with stabilized milling by-products. The combination of stabilizing treatments with roller milling, followed by flour re-combination/re-constitution and/or enrichment, could provide UWFs with a better nutritional and technological quality. Moreover, although it seems that the roller milling method could ensure better preservation of the wheat's nutritional value (Jones, Adams, Harriman, Miller, & Van der Kamp, 2015), the consumer is still highly attracted by the "stone mill" label on flours or baked products, considering this method better than roller milling (Jones, Adams, Harriman, Miller, & Van der Kamp, 2015). This points out that it is pressing to improve the dissemination of scientific knowledge to the final target.

The presence of WB and WG significantly changes the chemical composition of flour. Regardless of the amount of milling by-products added, UWFs show a low endosperm fraction and include specific flour constituents, such as fibre, oil, enzymes, reactive components and anti-nutrients. The amount of the above constituents can change markedly, depending on various factors: (i) level of addition, (ii) milling by-product treatment (if any), (iii) milling process, (iv) bran stream fraction (v) wheat species and cultivar, and (vi) environmental conditions.

Considering WG, the main constraints associated with its utilization in the baking industry are represented by its poor chemical stability, the presence of reducing compounds (glutathione) that degrade breadmaking ability, and the presence of non-polar lipids which tend to destabilize gas cells (Tebben et al., 2018; Boukid, Folloni, Ranieri, & Vittadini, 2018).

On the other hand, flour supplementation at different levels and/or with different bran fractions has shown deleterious impacts on the technological properties of doughs and breads (Hemdane et al., 2016). Overall, flours containing bran produce a poor loaf volume, dark colour, dense and firm texture, and bitter taste (Hemdane et al., 2016). The following reasons account for these negative effects: fibre-gluten interactions, dilution of gluten proteins by non-endosperm proteins, fibre competition for water resulting in insufficient hydration of gluten and starch, physical effects of bran particles and bran constituents on the gluten network and high level of ferulic acid (Tebben et al., 2018).

3.1.1 Unprocessed UWFs

The incorporation of raw milling by-products in flour without introducing some modification of the product formula and/or processing method gives "unprocessed UWF". It generally shows a poor breadmaking performance. However, some studies have introduced raw milling by-products into the flour while maintaining an acceptable bread quality (Table 2).

Banu, Stoenescu, Ionescu, & Aprodu (2012) investigated the addition of bran streams (3%, 5%, 10%, 15%, 20%, 25%, 30% expressed on total flour weight) to refined flour on dough rheology and bread quality. Despite the negative effect of ash on the properties of flour and on bread quality, the incorporation of 25% bran streams showed the same ash content as UWF, but dough rheology and bread physical parameters were significantly improved. Therefore, this level of incorporation can be used to increase the nutritional value of the breads with less damage on the bread quality compared to wholewheat flour.

Blandino et al. (2013) tested refined flour enriched with selected fractions obtained by sequential pearling of wheat kernels and added at 5 different levels (5%, 10%, 15%, 20%, 25% expressed on total flour weight) on dough rheology, bread quality and nutritional properties. The presence of a 10% pearled fraction enhanced the nutritional value of the bread, revealing only a slightly increase in deoxynivalenol contamination and showing a technological quality comparable to the control.

Bagdi et al. (2015) evaluated the breadmaking potential of an aleurone-rich flour (ARF) (40g/100g, 75g/100g) in comparison with refined bread. The ARF was suitable for breadmaking without any flour additives. Bread made with aleurone-rich flour showed better nutritional properties than refined bread, but a low technological quality. The optimal blending ratio for the sensory quality resulted 40g/100g since it showed a similar acceptability to the control sample.

Sun, Zhang, Hu, Xing, & Zhuo (2015) tested different levels (0%, 3%, 6%, 9%, 12% expressed on total flour weight) of WG flour to improve the quality of Chinese steamed bread (CSB). WG negatively affected both the dough and bread properties, and the steaming performance, in proportion to the level of addition. The incorporation of up to 6% WG showed fewer negative effects and gave CSB with acceptable sensory characteristics, suggesting that this blend could be used for the production of functional breads.

Pasqualone et al. (2017) compared the effect on breadmaking ability of two substitution levels (100g/1kg, 200g/1kg) of three different durum wheat milling by-products: i) residuals of the second and third debranning steps (DB), ii) the micronized and air-classified thin fraction from the same residuals (MB), or iii) coarse bran from roller milling of non-debranned durum wheat (B). MB and DB did not alter the textural properties compared to B. Furthermore, the addition of MB (100g/1kg) improved the nutritional value of the bread without reducing its quality. Hence, debranning followed by micronization could represent an interesting strategy for UWF breads from durum wheat.

Literature reference	Wheat flour	Milling by-product supplement	Supplementation with good bread technological and sensory quality
Banu et al. (2012)	Refined flour	Bran streams (3%, 5%, 10%, 15%, 20%, 25%, 30% expressed on total flour weight)	25% bran stream
Blandino et al. (2013)	Refined flour	Pearled fractions (5%, 10%, 15%, 20%, 25% expressed on total flour weight)	10% pearled fraction
Bagdi et al. (2015)	Refined flour	Aleurone (40%, 75% expressed as percentages of refined flour)	40% (only on sensory profile)
Sun et al. (2015)	Refined flour	Wheat germ (0%, 3%, 6%, 9%, 12% expressed on total flour weight)	6% wheat germ
Pasqualone et al. (2017)	Refined flour (re-milled semolina)	3 fractions of durum wheat milling by-products (10%, 20% expressed as percentages of refined flour): i) bran obtained from non-debranned wheat ii) second and third debranning fractions mixed together iii) thin subfraction obtained by micronization and air classification of the second and third debranning fraction mix	10% second and third debranning step

Table 2. The use of unprocessed unrefined wheat flours (UWFs) for breadmaking. Reporting literature reference, wheat flour, type of milling by-products used for supplementation and level of supplementation that gave good bread technological and sensory quality.

3.1.2 Processed UWFs

In the literature several treatments have been developed for wheat kernels and milling by-products, namely "processed UWF". The processing treatments that showed technological improvements for the use of processed UWFs in breadmaking are reported below (Table 3).

Literature reference	Treatment	Tested variables	Measurements	Main results
Ding et al. (2018)	Germination	Germination time	Hagberg falling number (FN) Rapid Visco Analyser (RVA) Starch pasting properties Mixolab mixing properties Physicochemical analysis γ-aminobutyric acid (GABA) content	Controlled germination (t=5-15 h, T= 28±2°C, RH=95±3%) improved wholewheat flour functionality
Ritcher et al. (2014)	Germination	Germinated wholewheat flour + vital gluten (0%, 3%, 4%, 5%) for breadmaking	Farinographic test Proof time Loaf volume Sensory analysis	100% germinated wholewheat bread showed better technological and sensory quality than control; Vital gluten did not improve bread quality
Zilic et al. (2016)	Germination	Germination effect on wholewheat protein functionality	Total and free sulfhydryl (-SH) groups Lipoxigenase (LOX) and peroxidase (POX) activity SDS-PAGE gel electrophoresis Total antioxidant capacity of albumin+globulin proteins Gliadin and glutenin immunogenicity Analysis of pasting viscosty	Total protein content did not change with germination Intensive protein hydrolysis Increased antioxidant capacity of albumin + globulin fraction and reduced glutenin antigenicity Potential health positive effects
Johnston et al. (2019)	Germination	Germination effect on wholewheat flour functionality and flavour	Kernel hardness Hagberg falling number (FN) Sodium dodecyl sulphate sedimentation analysis (SDS) Starch content Total dietary fibre Alfa-amylase activity Metabolite analysis Mixograph analysis Sensory analysis	Controlled germination (t=24 h, T=21°C, FN=200 s, excess of water) increased wholewheat bread volume and flavour

Table 3. References concerning the use of processed unrefined wheat flours (UWFs) produced through treatments on the raw material, i.e., wheat kernels. Outlining type of treatment, tested variables, measurements made and main results.

3.1.2.1 Pre-treatment of wheat kernels: germination

In recent years, several studies have investigated the impact of the germination process on the technological and nutritional quality of cereals, pseudo-cereals and pulses (Lemmens et al. 2019; Bellaio, Kappeler & Bühler, (2013); Richter, Christiansen, & Guo, 2014; Benincasa, Falcinelli, Lutts, Stagnari, & Galieni, 2019). In fact, by inducing the activation of hydrolytic enzymes for plant growth and development, germination leads to a considerable improvement in the product nutritional value, which makes this process attractive for healthy and functional foods. Applications of the germination process in breadmaking are reported in the literature, but most of these studies used refined wheat flours in the bread formula (Lemmens et al., 2019). The main issue of this approach is optimizing the processing conditions: longer germination times (>72 h) are required to improve the nutritional value of the flour, but they negatively impacted the flour technological performance; on the other hand, only shorter times (20-36 h) or low substitution levels (10%-20%) improved the technological properties of doughs and breads, although they showed lower effects on their nutritional value (Lemmens et al., 2019). These results reflect the different degrees of enzyme activities in wheat kernels as a function of the germination time. Activation of the proper alfa-amylase activity can promote yeast fermentation, carbon dioxide production and gas cell expansion, thus determining a higher oven spring and improving bread volume (Lemmens et al., 2019). Moreover, optimized alfa-amylase activity can also improve the product's shelf life and sensory quality (Lemmens et al., 2019).

Marti, Cardone, Nicolodi, Quaglia, & Pagani (2017) proposed the addition of a low amount of germinated wheat flour (1.5%) for a long amount of time (72-90 h) as a natural improver in breadmaking with refined flour. Germinated flour produced similar effects to common improvers (i.e., 0.5% malt or 0.5% enzymatic improver). It could be interesting to test the effect of this natural improver on the technological performance of UWF.

Richter, Christiansen, & Guo (2014) developed a 100% germinated white spring wholewheat flour for bread applications. The authors compared 100% white wholewheat flour (control) with 100% germinated white wholewheat flour, including different additions of vital gluten (0%-5%) in the bread formula (Richter, Christiansen, & Guo, 2014). Germinated wholewheat flour significantly increased loaf volume (5%-9%), independently of the presence of gluten (Richter, Christiansen, & Guo, 2014). Moreover, germinated flour significantly improved the sensory quality of the bread, reducing the bitter taste.

Zilic et al. (2016) investigated the effect of germination on wholewheat flour proteins. Intensive protein hydrolysis was revealed by an increase in free SH groups and a decrease in albumin + globulin polypeptides with a molecular weight of over 85.94 kDa and between 85.94 and 48.00 kDa. Although this modification affected the dough's viscoelastic properties, germinated wheat flour is proposed as a potential food ingredient owing to the high antioxidant capacity and reduced antigenicity of the glutenin fraction (Zilic et al., 2016).

Ding et al. (2018) tested germination time on the functionality of wholewheat doughs. Controlled germination for 5-15 h (T= 28±2°C and RH=95±3%) produced wholewheat flour with improved functionality: enhanced glucose content, reduced starch retrogradation during gelatinization, improved gluten quality, and increased dough stability during mixing (Ding et al., 2018).

Johnston et al. (2019) applied controlled germination in the production of wholewheat. Controlled germination (t=24 h, T=21°C, FN=200 s, excess of water) to increase the activity of α-amylase by decreasing the Falling Number (FN) from 350 s to 200 s reduced dough mixing time and increased bread specific volume (Johnston et al., 2019). Sensory analysis revealed a higher acceptability for germinated wholewheat breads, thanks to their lower degree of bitterness, greater sweetness and moisture (Johnston et al., 2019). Hence, germinating wholewheat flour to an FN value of 200 s proved to be a good strategy to improve flour functionality and consumer preference for wholewheat breads (Johnston et al., 2019).

A very recent work by Cardone, Marti, Incecco, & Pagani (2020) showed promising results on the application of a controlled germination to enhance the quality of wholewheat breads. In fact, under the conditions applied in this study (48h, 20°C, 90% relative humidity), although the decrease in dough rheological features, a significant improvement in gluten stretching ability and bread physical properties was obtained.

Further studies on the application of germination to improve the UWF breadmaking performance could be an interesting field of research. The substitution of refined flour with UWF may not require additional enhancement of the nutritional quality, since the raw material is naturally characterized by a high nutritional value.

3.1.2.2 Pre-treatment of milling by-products

Over time several pre-treatments have been developed for milling by-products to stabilize their chemical composition and better understand their effects on the breadmaking performance (Hemdane et al., 2016; Boukid, Folloni, Ranieri, & Vittadini, 2018). The present review reports the results of the most promising treatments on technological quality (Table 4).

Hemdane et al. (2016) extensively reviewed wheat bran pre-treatments. Chemical treatments did not improve bread quality; very little work has been performed on enzymatic treatments (Santala, Lehtinen, Nordlund, Suortti, & Poutanen, 2011; Messia et al. 2016), while the effects of bran particle size reduction and (hydro)thermal treatments on breadmaking still remain unclear. Pre-soaking appears to be a promising strategy for the incorporation of bran in breadmaking since it generally improved the bread quality (Messia et al. 2016); however, a complete understanding of this approach has yet to be established. In a recent work following the method proposed by Wang et al. (2015a, b), Zhang et al. (2019) developed an arabinoxylan-enriched flour (AXF) (ash content between 39.2%–55.8%) as a fibre supplement (2%, 5%, 10%) for refined bread. AXF pre-soaking positively affected flour functionality, resulting in bread with comparable properties to the control (Zhang et al., 2019). Fermentation treatment has been reported to enhance breadmaking ability: it was effective in improving bread volume and crumb softness.

The impact of WG supplementation on breadmaking is reviewed by Boukid, Folloni, Ranieri, & Vittadini (2018). The rheological properties of doughs were affected by the different WG treatments. Fermentation by lactic acid bacteria appeared to be the most promising treatment, showing a significant improvement in the dough properties. In all cases, the addition of more than 20% WG severely damages dough quality. Considering bread quality, the addition of up to 5% extruded WG increased bread volume and decreased bread firmness. Sourdough fermentation of WG positively affected bread quality, by decreasing crumb firmness, resilience and fracturability, and enhancing bread shelf life without reducing product acceptability (Boukid, Folloni, Ranieri, & Vittadini, 2018).

These results revealed that the use of sourdough fermentation on milling by-products enhanced the performance of UWF in breadmaking. Indeed, Gobbetti, Rizzello, Di Cagno, & De Angelis (2014) extensively reported the positive effects of using this approach for wholegrain products. Pre-fermentation allowed modification of the technofunctionality of the milling by-products, showing improved technological quality in terms of dough retention capacity, loaf volume and crumb softness during storage. In addition, it decreased the anti-nutritive factors and enhanced the sensory properties.

Recently, Pontonio et al. (2020) proposed an integrated biotechnological approach, combining LAB fermentation with xylanase treatment on milling by-products (Pontonio et al., 2020). Biochemical and nutritional analysis revealed that fortified breads had higher protein digestibility and a lower glycemic index combined with a better sensory quality (Pontonio et al., 2020). Therefore, a significant improvement could be achieved in UWF breads by applying an integrated approach, suggesting new strategies for the exploitation of UWF.

The reviewed studies identified effective technological strategies for producing UWF with a high technological quality. The possibility of supplementing bread with even low amounts of milling by-products, without decreasing the bread quality, should be regarded as a technological success.

Literature reference	Milling by-product	Treatments	Best results on bread quality	Interpretation of results
Hemdane et al. (2016)	Wheat bran	Pre-soaking Particle size reduction	14% pre-soaked bran 22% pre-soaked, fine-ground bran	Reduction of bran water uptake during mixing
Hemdane et al. (2016)	Wheat shorts	Pre-soaking	Bread specific volume	Activation of endogenous lipoxygenase
Hemdane et al. (2016)	Wheat bran	Pre-soaking (limited/excess water)	Good bread quality	A complete understanding has not been established yet. Some hypotheses proposed: -Saturating bran with water before mixing prevent the detrimental effects on dough/bread quality; -Activation of endogenous lipoxygenase oxidize components detrimental to bread quality -Washout effect when pre-soaking in excess of water
Messia et al. (2016)	Wheat bran	Pre-soaking Enzyme addition (xylanase, amylase, cellulase)	Improved dough rheology and bread physical properties	Modification of arabynoxylan solubility allowing a better redistribution of water
Zhang et al. (2019)	Arabinoxylan flour from wheat bran	Pre-soaking Enzyme addition (xylanase)	Up to 10% pre-soaked arabinoxylan flour	The positive effects associated to the pre-soaking of arabinoxylan flour was not explained
Hemdane et al. (2016)	Wheat bran (native bran and bran from peeled kernel)	Fermentation (20 h, yeast starter)	20% fermented bran from peeled kernel	Solubilization of arabinoxylans Reduction of endogenous xylanase activity
Hemdane et al. (2016)	Wheat bran	Fermentation (8 h, lactic acid bacteria and yeast strain) Particle size reduction	15% 160 um bran fermented 8 h	Lactic acid fermentation (Lactobacillus brevis)
Boukid et al. (2018)	Wheat germ	Sourdough fermentation (bacteria and yeast)	Up to 20% sourdough fermented wheatgerm	Reduction of enzymatic activities (lipase, lipoxygenase) Reduction of glutathione content
Boukid et al. (2018)	Wheat germ	Sourdough fermentation (Lactobacillus plantarum LB1, Lactobacillus rossiae LB5)	Better nutritional, chemical and stabilization properties	Reduction of pH Reduction of enzymatic activities (lipase, lipoxygenase) Higher total amino acids Higher protein digestibility Inactivation of anti-nutritional factors Higher antioxidant activity
Boukid et al. (2018)	Wheat germ	Sourdough fermentation (Lactobacillus plantarum LB1, Lactobacillus rossiae LB5)	4% sourdough fermented wheat germ	Reduction of enzymatic activities (lipase, lipoxygenase) Reduction of glutathione content
Boukid et al. (2018)	Wheat germ	Sourdough fermentation (Lactobacillus plantarum LB1, Lactobacillus rossiae LB5	4% sourdough fermented wheat germ bread shelf life	Antifungal activity of sourdough fermented wheat germ (phenolic acids, organic acids) Lower pH values

Table 4. References concerning the use of processed UWF produced through treatments on the raw material, i.e., milling by-products (wheat bran and wheat germ). Summarizing milling by-product, type of treatment, best results on bread quality and interpretation of the main results.

3.2 Bread formulation and improvers for UWF

In the literature the most common solution to improve the breadmaking performance of UWF and, consequently, the quality of UWF breads is to modify the bread formula; the literature results are outlined in Table 5.

3.2.1 Optimization of water amount for UFW breads

Some improvements in breadmaking with UWF can be obtained by optimizing the amount of water in the bread recipe (Table 5). Cappelli et al. (2018) examined the effect of water (70%, 76%, 82%, 88%, 94% expressed as a percentage of the dry weight of the flour) and degree of flour refinement (refined, brown and wholewheat flour) on the dough rheology. Significant differences in rheological properties were found for refined flour compared to UWF, showing that alveographic analysis cannot be extended to unrefined doughs. Addition of the optimal amount of water, modelled in function of the degree of flour refinement, could be a strategy to optimize the rheological parameters relating to product quality: flour strength "W" and the ratio between tenacity "P" and extensibility "L", P/L (Cappelli et al., 2018). This approach could improve UWF dough quality, without introducing additional ingredients to the recipe.

Similar results were reported in a survey conducted by Guerrini, Parenti, Angeloni, & Zanoni (2019) on the breadmaking process with UWF. The creation of highly hydrated doughs improves the flour workability and bread quality (Guerrini, Parenti, Angeloni, & Zanoni, 2019). Indeed, in the literature it is known that the inclusion of bran significantly affects the water adsorption capacity of the flour and causes a competition for the water uptake with the other flour constituents (Hemdane et al., 2016). High water quantities could allow proper hydration of the gluten matrix even in the presence of bran, resulting in a better P/L balance as well as a higher W.

All these results may derive from the wide utilization of the Farinographic test as the official predictor of the water absorption of the flour: this evaluation works well for refined flour, but it is not suitable for UWF (Bruckner et al., 2001; Schmiele, Jaekel, Patricio, Steel, & Chang, 2012; Hemdane et al., 2016).

3.2.2 Modification of the flour for UWF bread

In the literature, few process strategies have tested a more "natural approach" to the use of improvers: the addition of UWF flour in a modified form, as reported in Table 5. Parenti et al. (2019) reported an improvement in breadmaking performance with the use of pre-gelatinized brown flour (6%). Pre-gelatinized UWF was obtained by heating some of the bread dough flour to 85°C in water; the product was cooled to room temperature and tested on the dough and bread properties. The addition of the flour in a different physical form increased the water absorption capacity, improved the alveographic parameters, and increased the bread volume, crumb softness and shelf life (Parenti et al., 2019).

Hung, Maeda, & Morita (2007) tested the addition of whole waxy flour in order to improve the quality of high-fibre bread. Different levels of whole waxy flour were used to substitute refined flour (10%, 30%, 50% expressed on total flour weight); the resultant flour mixtures were tested on the breadmaking performance compared to refined flour. This strategy improved crumb softness during bread storage (Hung, Maeda, & Morita, 2007). It could be interesting to investigate the use of whole waxy flour compared to 100% wholewheat flour for breadmaking, in order to evaluate the impact of this ingredient on the quality of wholewheat bread.

3.2.3 Improvers for UWF bread

The use of improvers in breadmaking with UWF is summarized in Table 5. Tebben et al. (2018) reviewed the effects of common bread improvers, namely enzymes, emulsifiers, hydrocolloids, oxidants and other functional ingredients on the performance of wholewheat flour. A positive role is outlined for some enzymes: (i) by hydrolysing arabinoxylans (AX), xylanase was reported to decrease the water absorption of the flour, increase the concentration of fermentable sugars in the dough, the rate of fermentation and the dough proof height; moreover, xylanase improved the gas retention capacity, loaf volume, crumb softness and crumb staling; (ii) alfa-amylase appeared beneficial under certain conditions; (iii) G4-amylase showed promising effects on loaf volume, crumb hardness and staling (Tebben et al., 2018).

Considering hydrocolloids, a general improvement in dough rheology is reported in the literature (Tebben et al., 2018; Farbo et al., 2020). The effects of hydrocolloids change in function of their typology and level of addition. With regard to bread dough, the use of carboxymethylcellulose (CMC) decreased the final proof time and resistance to extension. Guar gum (GG) combined with an emulsifier (diacetyl tartaric esters of monoglycerides, DATEM) was reported to increase the fermentation stability and slightly increased bread volume. However, both CMC and GG reduced the elasticity of wholewheat dough. Hydroxypropyl methylcellulose (HPMC) increased dough elasticity, proof height, and decreased resistance to extension (Tebben et al., 2018).

Farbo et al. (2020) studied the effect of methylcellulose (MC), GG, psyllium gum (PG), xanthan gum (XG) and tara gum (TG) on the quality of dough made with old durum wheat. They found that 1% of PG or XG improved dough extensibility, while all hydrocolloids increased gas retention.

Considering bread quality, GG was able to increase the specific volume of wholewheat bread. Furthermore, a non-significant effect of HPMC, XG and dextran was reported on bread volume. Conversely, HPMC proved effective in increasing the specific volume of both refined and wholewheat bread, while CMC did not improve the loaf volume of either variety of bread (Tebben et al., 2018).

Oxidants are commonly added in breadmaking to increase dough strength by forming disulphide bonds through the oxidation of free sulfhydryl groups on the gluten proteins (Zhou et al., 2014). The presence of reducing compounds in wholewheat flour counteracts the effect of oxidants, which must be added at higher levels (Tebben et al., 2018). Hence, higher amounts of oxidants will also presumably be required for other UWF typologies. Potassium bromate and ascorbic acid improved the dough rheology, dough strength and gas retention ability. The addition of rosehip as a source of ascorbic acid increased the resistance to extension and reduced the extensibility of the wholewheat dough. The best effect on bread volume was reported for ascorbic acid, added at 200 ppm; accordingly, rosehip resulted effective in enhancing bread volume. This latter improver also improved the sensory score of the crumb, increasing the acceptability of the wholewheat bread. Conversely, potassium bromate showed little effect on loaf volume (Tebben et al., 2018).

Emulsifiers in breadmaking cause dough strengthening and/or crumb softening (Tebben et al., 2018). The addition of DATEM was reported by some studies to increase the fermentation stability, whereas the opposite effect was observed by others (Tebben et al., 2018). However, these studies are consistent in showing that DATEM improved dough elasticity, a valuable property for the breadmaking performance. Another emulsifier, sodium stearoyl lactylate (SSL), improved the handling properties of the wholewheat dough (Tebben et al., 2018).

The specific volume of wholewheat bread was generally improved by the addition of emulsifiers (Tebben et al., 2018). DATEM was reported to produce positive effects. Furthermore, the combined addition of DATEM and oxidants improved the gas-holding ability of the dough during the proofing and baking phases. DATEM and SSL had the greatest effect on volume increase, but ethoxylated monoglycerides, succinylated monoglycerides and lecithin significantly increased loaf volume too. On the other hand, polysorbate and monoglycerides did not affect the parameter. Similar results were reported for the inclusion of DATEM, SSL, soy lecithin, polyoxyethylene sorbitan monostearate (polysorbate-60), poly-oxyethylene sorbitan monopalmitate (polysorbate-40) and glycerol-monostearate: all these emulsifiers increased the volume of the wholewheat bread. Conversely, the addition of monoglycerides, DATEM and SSL was not effective in improving wholewheat or refined bread specific volume (Tebben et al., 2018).

DATEM and mono- and diglycerides were reported to improve the crumb structure of wholewheat bread; a similar effect was observed with SSL as well as an increase in the eatability score (Tebben et al., 2018).

The supplementation of vital gluten is effective in overcoming the multiple problems related to wholewheat bread (Tebben et al., 2018).

Parenti, Guerrini, Cavallini, Baldi, & Zanoni, (2020) tested the addition of 7 improvers (i.e., sucrose, sodium chloride, extra virgin olive oil, gelatinized flour, GG, ascorbic acid and ice) to optimize the quality of wholewheat bread. The optimized sample resulted from the combination of sucrose (2%) and extra virgin olive oil (3%), disclosing the interesting role that these improvers can play in the quality of wholewheat bread (Parenti, Guerrini, Cavallini, Baldi, & Zanoni, 2020). Furthermore, the authors proposed a two-step optimization approach for improving the use of UWF in breadmaking: (i) the Screening Design method revealed the most relevant factors affecting bread quality; (ii) the Full Factorial Design gave an in-depth evaluation of the selected variables and allowed identification of the optimized sample (Parenti, Guerrini, Cavallini, Baldi, & Zanoni, 2020).

All these results concerned the use of improvers on wholewheat flour. However, due to the very different composition of the raw materials which probably changes the effects of the improvers, they should be further tested before extending these findings to all UWF breads.

Literature reference	Process strategy	Tested variables	Wheat flour	Improvement of bread quality
Cappelli et al. (2019)	Optimization of water amount	Water amount (70%, 76%, 82%, 88%, and 94%) Flour refinement degree (refined, brown, wholewheat)	Refined, brown and wholewheat flour	Optimal water addition as a function of degree of flour refinement
Guerrini et al. (2019)	Optimization of water amount	Different variables used by bakers	Brown and wholewheat flour	Higher water amount
Parenti et al. (2019)	Modification of the flour	Water amount (59%, 70%, 80%) Pre-gelatinized flour (0%, 6%)	Brown flour	6% pre-gelatinized flour + high water amount
Hung et al. (2007)	Modification of the flour	Waxy wholewheat flour (0%, 10%, 30%, 50%)	Wholewheat flour and waxy wholewheat flour	Waxy wholewheat breads showed softer crumb during storage
Tebben et al. (2018)	Improver	Enzyme, xylanase	Wholewheat flour	Optimization of xylanase usage level
Tebben et al. (2018)	Improver	Enzyme, alfa-amylase	Wholewheat flour, blends of refined and wholewheat flours	Optimization of amylase usage level
Tebben et al. (2018)	Improver	Enzyme, G4-amylase	Wholewheat flour	Optimization of G4-amylase usage level
Tebben et al. (2018)	Improver	Hydrocolloids (carboxymethylcellulose, CMC; guar gum, GG; methylcellulose, MC; psyllium gum, PG; xanthan gum, XG; tara gum, TG)	Wholewheat flour	0.5%-1% hydrocolloids
Tebben et al. (2018)	Improver	Oxidants (potassium bromate, ascorbic acid, rosehip as a source of ascorbic acid)	Wholewheat flour	Optimum amount of antioxidants corresponds to higher quantities than refined flour; best results with 200 ppm
Tebben et al. (2018)	Improver	Emulsifiers (diacetyl tartaric esters of monoglycerides, DATEM; sodium stearoyl lactylate, SSL; ethoxylated monoglycerides, succinylated monoglycerides, lecithin, polyoxyethylene sorbitan monostearate, poly-oxyethylene sorbitan monopalmitate, glycerol-monostearate, mono- and diglycerides)	Wholewheat flour	Usage level 0.4%-0.5% Positive results emulsifiers combined with oxidants
Tebben et al. (2018)	Improver	Vital gluten	Wholewheat flour	Usage level 2%-2.5%

Table 5. Literature references about the modification of unrefined wheat flours (UWFs) bread formula to improve breadmaking performance. Reporting the main process strategies (i.e., optimization of water amount, modification of flour and addition of improvers), tested variables, type of UWF and strategies that improved bread quality. Percentages relate to flour base (ingredient/total flour %).

3.3 The breadmaking process with UWFs

Only a few studies have investigated the possibility of modifying the breadmaking operating conditions. The breadmaking process has been designed to maximize the quality of refined bread. Therefore, the substitution of refined flour with UWF may require an adaptation of the process to the different characteristics of the raw material. Processing conditions, such as the type of mixer, mixing time and speed, resting period etc., may require modifications from the standard procedure. This latter area of research appears poorly investigated in the literature, since the greatest efforts have been made in modifying the bread formulation, while the breadmaking variables were kept almost unchanged. Studies about modifications of the breadmaking process with UWFs are reported in Table 6.

3.3.1 Mixing

The mixing is one of the most important phases in the breadmaking process since most of the characteristics of the final product are determined during this phase (Zhou et al., 2014). Considering the different composition of UWF, modification of the mixing variables (type of mixers, mixing speed, mixing time…) could represent a good strategy to be explored, despite being poorly investigated in the current literature.

Angioloni & Rosa (2006) tested the effect of mixing time (10-15-20 s) combined with an improver (cysteine, 20 mg/kg) on the rheological properties of refined and wholewheat dough obtained at high-speed revolutions (1600 rpm). Dough viscoelastic behaviour was affected by both cysteine and kneading conditions. Cysteine significantly reduced the mixing time (optimum = 15 s) by decreasing the elastic component of the dough and aiding dough relaxation in both refined and wholewheat flour. Therefore, the use of high-speed mixing combined with cysteine could be useful to improve UWF doughs.

Parenti et al. (2013) tested different mixing times on the breadmaking of brown flour. Two trials evaluated different mixing times ((i) 12, 17, 22 min; (ii) 17, 22, 27 min). Mixing time significantly affected loaf increase during proofing: the samples mixed for 17 min showed the highest value in both trials. Furthermore, doughs obtained at the optimum mixing time (17 min) were characterized by a better water retention capacity during storage.

The control of the mixing time also proved to be extremely important in the survey by Guerrini, Parenti, Angeloni, & Zanoni (2019); short times, between 10 and 20 min, represented one of the most effective strategies for the breadmaking process with UWF.

A recent work by Cappelli, Guerrini, Cini, & Parenti (2019) investigated the delayed addition of bran and middlings during the mixing step. Three bran and middlings substitution levels (10%, 20%, 30% expressed on refined flour weight) and five times of addition (0, 2, 3.5, 5, 6.5 min) were tested on the dough rheology and bread quality. The addition of bran and middlings at 2 min into the mixing step improved the dough rheology and increased the bread specific volume. Furthermore, the combination of 10% bran and middlings with time of 2 min produced bread of a better quality than the control bread (i.e., without delayed addition).

A specific laboratory test, developed to predict the optimized mixing time, could boost the research on the mixing step, but, to the best of the author's knowledge, no such test currently exists.

3.3.2 Leavening

During leavening, the bread loaf develops its final structure and several modifications of its constituents occur as a function of the different leavening agents used in the recipes. Sourdough fermentation represents one of the oldest biotechnologies in cereal food production; however, when industrial-scale baking was developed in the 19th century, baker's yeast – Saccharomyces cerevisiae – became the most common leavening agent (Zhou et al., 2014). In recent years, the increasing interest in healthy and functional foods has led to a rediscovery of sourdough bakery products, which are characterized by positive health benefits and unique flavours (Zhou et al., 2014). Chavan & Chavan (2011) made an exhaustive review of this ancient biotechnology. In the present review, only the issues related to the technological performance of UWF are discussed. In the literature it is largely reported that the substrate, mainly flour, used for sourdough production deeply influences its properties (Chavan & Chavan, 2011; Decock, & Cappelle, 2005). The presence of bran, increasing the ash content of wheat flour, promotes the growth of lactic acid bacteria (LAB) and increases the acidification of the sourdough system. LAB are responsible for the production of several organic acids, which are reported to improve the swelling of gluten and increase gas retention, while functioning as natural dough conditioners and reducing bread staling. Furthermore, the acid enhances the solubility of the glutenin fraction, improving the swelling power of the gluten (Chavan & Chavan, 2011). Hence, the use of UWF for sourdough production seems to improve the breadmaking performance, thanks to a better development of the gluten matrix. Studies on UWF performance are reported in Table 6.

In the survey by Guerrini, Parenti, Angeloni, & Zanoni (2019), all of the bakers use sourdough as the leavening agent for breadmaking with UWF: they perceive that this method improves the quality of the final product.

Komlenić et al. (2010) showed the positive effects of biological acidification on the quality of bread obtained with refined and wholewheat flour. They investigated the effect on dough and bread properties of three different acidifications: chemical (lactic acid) and biological (dry sourdough and Lactobacillus brevis pre-ferment) acidification. The bread specific volume was only significantly increased by the biological acidifiers, whereas the acidifier typologies improved the crumb hardness (Komlenić et al., 2010). Therefore, dry sourdough, characterized by a longer shelf life and better stability, could be an interesting strategy for breadmaking with UWF.

Taccari et al. (2016) reported the possibility of applying the back-slopping technique to produce type I sourdough from wholewheat flour. Wholewheat sourdough improved the quality of high fibre breads, overcoming the detrimental effect of bran on bread volume. Moreover, sourdough fermentation improved bread texture, flavour, nutritional value and shelf life. The study outlines the suitability of wholewheat flour for sourdough production, encouraging further research for its application in UWF breadmaking.

Choi, Kim, Hwang, Kim, & Yoon (2005) evaluated the application of Leuconostoc citreum HO12 and Weissella koreensis HO20 isolated from kimchi as starter cultures for sourdough wholewheat bread. The sourdoughs fermented with the selected LAB had an optimal Fermentation Quotient (FQ), a criterion for good bread quality. Although no significant improvement was observed on bread specific volume, the LAB reduced crumb hardness on both fresh and stored breads (Choi, Kim, Hwang, Kim, & Yoon, 2005). Hence, the study presented the potential application of LAB isolated from kimchi for the improvement of UWF bread quality.

Didar et al. (2011) observed positive effects on bread quality (95% extraction rate) and sensory properties upon performing sourdough fermentation with Lactobacillus plantarum (PTCC 1058) and Lactobacillus reuteri (PTCC 1655). Different dough yields (DY, 250 and 300) and different levels of sourdough addition (10%, 20%, 30%) were also tested. Lb. plantarum sourdough with a DY of 250 and 30% addition produced the greatest effect on the overall quality score of the breads (Didar et al., 2011).

Katina, Heiniö, Autio, & Poutanen (2006a) studied the influence of sourdough conditions on bread flavour and texture. Ash content (0.6-1.8 g/100 g), fermentation temperature (16-32°C), and fermentation time (6-20 h) were considered independent factors and different starter cultures (i.e., Lactobacillus plantarum, Lactobacillus brevis, Saccharomyces cerevisiae or a combination of yeast and LAB) were tested. Ash content and lactic acid fermentation were the main factors affecting the intensity of the sensory attributes. The greater the ash content, the higher the intensity of both desired and undesired flavour attributes. An optimization of the process conditions, according to the ash content and the specific LAB strain, improved the sensory quality of UWF breads. However, the improvement of bread volume and texture required different optimized conditions than those required for bread flavour. Hence, an efficient use of sourdough fermentation has to consider its end use in wheat baking (Katina, Heiniö, Autio, & Poutanen, 2006a).

The results from the use of sourdough as a leavening agent showed positive effects on UWF bread quality. The drawback of this procedure is primarily represented by the great variability of the sourdough composition, which makes the process difficult to standardize. Further research is needed to find new solutions to combine the use of this leavening agent with a standardization of bread features.

3.3.3 Baking

Baking is the final step in the breadmaking process. The phenomena occurring during this phase include gas evaporation, starch gelatinization, modification of the bread loaf from a sponge-like to a porous structure, and water evaporation. The most significant factors of the baking step are represented by temperature, time and moisture (Zanoni, Peri, & Pierucci, 1993; Zanoni, Pierucci, & Peri, 1994; Zhou et al., 2014).

In the literature there appears to be a lack of information on the baking step specifically developed for UWF breads. Guerrini, Parenti, Angeloni, & Zanoni (2019) reported that bakers create high temperatures at the beginning, followed by a temperature decrease, to improve the quality of UWF breads. Moreover, the majority of bakers check the moisture during this step, since it represents another critical factor affecting bread quality. In fact, especially in the first phase of baking, the addition of moisture improves loaf expansion. Therefore, modification of the baking conditions, such as temperature and moisture, in function of the characteristics of the raw material could represent another interesting field of exploration.

Literature reference	Breadmaking step	Tested variable	Wheat flour	Processing strategy
Angioloni et al. (2006)	Mixing	Mixing time (10, 15, 20 min) Cysteine (20 mg/kg)	Refined and wholewheat flour (T. aestivum L.)	Combination of high-speed mixer and cysteine addition
Parenti et al. (2013)	Mixing	Mixing time (12, 17, 22, 27 min)	Brown flour (T. aestivum L.)	Optimized mixing time (17 min)
Guerrini et al. (2019)	Mixing	Different variables used by bakers	Brown and wholewheat flour (T. aestivum L., T. durum)	Short mixing time (10-20 min)
Cappelli et al. (2019)	Mixing	Time for addition of bran and middlings during mixing (0, 2, 3.5, 5, 6.5 min) Levels of bran and middlings (10%, 20%, 30%)	Refined flour enriched with bran and middlings (T. aestivum L.)	10% bran and middlings added at t=2 min
Kolmenic et al. (2010)	Leavening	Biological acidification (dry sourdough, Lactobacillus brevis preferment) Chemical acidification (lactic acid)	Refined flour Wholewheat flour (commercial blends)	Biological acidification (dry form)
Taccari et al. (2016)	Leavening	Back-slopping technique for type I sourdough	Wholewheat flour (T. aestivum L.)	Application of back-slopping techniques for sourdough fermentation
Choi et al. (2005)	Leavening	Selected LAB isolated from kimchi as starter cultures (Leuconostoc citreum HO12 and Weissella koreensis HO20)	Wholewheat flour (T. aestivum L.)	Applicability of selected LAB (Leuconostoc citreum HO12 and Weissella koreensis HO20)
Didar et al. (2011)	Leavening	Sourdough fermentation with Lactobacillus plantarum (PTCC 1058) and Lactobacillus reuteri (PTCC 1655) Level of sourdough addition (10%, 20%, 30%) Dough yield (250 and 300)	Flour with 95% extraction rate (cv Alvand wheat)	30% Lb. plantarum sourdough with DY 250
Katina et al. (2006a)	Leavening	Sourdough time = 6-20 h Sourdough temperature = 16-32°C LAB and yeast for sourdough fermentation (Lactobacillus plantarum, Lactobacillus brevis, Saccharomyces cerevisiae or a combination of yeast and LAB) Flour ash content (0.6-1.8 g/100 g)	Flours with different ash content (0.6-1.8 g/100 g) (commercial flours)	Different flour ash contents required different optimization strategies

Table 6. Literature references about processing strategies for breadmaking with unrefined wheat flour (UWF). Outlining breadmaking step, tested variable, types of UWF used and process strategy.

3.4 UWF bread storage

The most important phenomena limiting the shelf life of breads are bread staling and microbial growth (Fernandez, Vodovotz, Courtney, & Pascall, 2006). Bread staling is a complex phenomenon, whose mechanism has not been well established yet; however, the most important factors seem to be starch retrogradation, starch-gluten interaction and moisture redistribution (Fadda, Sanguinetti, Del Caro, Collar, & Piga 2014; Curti, Carini, Tribuzio, & Vittadini, 2015). Bread microbial spoilage is generally caused by moulds, bacteria and yeasts (Melini, & Melini, 2018). Different approaches have been developed to reduce bread staling and microbial spoilage, which generally achieve positive effects, allowing the production of breads with a shelf life of up to 4 weeks (Fadda, Sanguinetti, Del Caro, Collar, & Piga 2014; Sargent, 2008). Hence, bread flavour and aroma have become the new limiting factors for bread shelf life.

3.4.1 Improving the shelf life of UWF bread

Different strategies can be applied to extend bread shelf life: (i) direct approach on the food matrix; (ii) indirect approach through packaging systems.

Within the direct approaches, Gobbetti, Rizzello, Di Cagno, & De Angelis (2014) reviewed the importance of fermentation of the raw material for wholegrain products. Specifically, the application of this method on milling by-products before their incorporation in the bread formula was reported to improve crumb softness during bread storage.

Furthermore, the germination process also showed positive effects on bread storage, linked to the activation of alfa-amylase activity (Lemmens et al., 2019).

The supplementation of pre-gelatinized UWF in the bread formula delayed bread staling, in terms of crumb specific volume and texture parameters (Parenti et al., 2019).

With regard to improvers, different enzymes reduce the staling of UWF bread: (i) xylanase and (ii) alfa amylase result the most effective enzymes; furthermore, one study has reported that (iii) G4-amylase showed a positive outcome, but further research is necessary to confirm this result (Tebben et al., 2018).

The effects of emulsifiers on wholewheat breads were reported by Tebben et al. (2018). DATEM showed anti-staling properties. A reduction in hardness was also reported for wholewheat bread with 0.4% DATEM or 0.6% monoglycerides. Similarly, 0.5% SSL was able to decrease the staling rate of wholewheat bread over 4 days of storage. It is interesting to note that DATEM and SSL only acted as crumb softeners in wholewheat breads but not in refined breads (Tebben et al., 2018).

The use of hydrocolloids in UWF breads led to controversial results. Both CMC and GG reduced the staling rate of wholewheat bread; HPMC softened the crumb of both wholewheat bread and refined breads, while another study reported that CMC inclusion was ineffective for both bread typologies. Furthermore, the literature reported that dextran and HPMC produced a non-significant reduction in the initial loaf hardness and delay in bread staling. Hence, further research is necessary to better understand the role of emulsifiers on UWF bread staling (Tebben et al., 2018).

Malted wholewheat flour in breadmaking reduced the staling of wholewheat bread (Tebben et al., 2018).

Some efforts have been made to increase oxidative stability during the storage of UWF breads, delaying rancidity phenomena.

Jensen, Ostdal, Skibsted, & Thybo (2011b) tested three antioxidants, alfa-tocopherol and fat-soluble and water-dispersible rosemary extracts, on the sensory profile and antioxidant capacity of wholewheat bread during storage. These antioxidants did not improve the sensory quality or stability of the wholewheat bread (Jensen, Ostdal, Skibsted, & Thybo, 2011b). Furthermore, alfa-tocopherol produced fresh wholewheat bread with higher concentrations of hydroperoxides and secondary lipid oxidation products, similarly to the stored control sample (Jensen, Ostdal, Skibsted, & Thybo, 2011b). Hence, lipid oxidation is responsible for less favourable sensory notes like a rancid aroma and flavour, bitter taste and astringency, attributes most often associated with low product acceptability (Jensen, Ostdal, Skibsted, & Thybo, 2011b).

Ning, Hou, Sun, Wan, & Dubat (2017) tested green tea powder (GTP) on the quality and antioxidant activity of wholewheat dough and bread. Five levels of GTP were tested (0 g, 1 g, 2 g, 3 g, 4 g/100 g flour): the higher the amount of GTP included, the worse the bread quality, while the antioxidant activity showed a reverse trend (Ning, Hou, Sun, Wan, & Dubat, 2017). The best result was obtained with GTP 1 g/100 g, since it did not affect bread quality while enhancing the antioxidant capacity (Ning, Hou, Sun, Wan, & Dubat, 2017). Hence, 1 g/100 g GTP resulted an effective improver in reducing the rate of peroxide accumulation in wholewheat bread during storage (Ning, Hou, Sun, Wan, & Dubat, 2017).

Lu, & Norziah (2011) studied the effect of substituting shortening with different levels of microencapsulated n-3 polyunsaturated fatty acid (PUFA) powder (1%, 1.75%, 2.5% of total dough weight) on the sensory and oxidative stability of UWF bread during storage. The flour used was a blend of wholewheat and refined flour (Lu, & Norziah, 2011). Breads containing PUFA were no different to the control containing shortening, revealing that PUFA had a similar effect on bread quality (Lu, & Norziah, 2011). The lowest PUFA addition (1%) resulted in bread with the best sensory acceptability for up to 3 days of storage, suggesting that this improver could be an effective substitute for shortening (Lu, & Norziah, 2011).

With regard to the breadmaking process, the most effective variable in enhancing the shelf life of UWF bread was sourdough fermentation (Chavan & Chavan, 2011; Taccari et al., 2016; Choi, Kim, Hwang, Kim, & Yoon, 2005).

The evaluation of the sensory profile of wheat bread during shelf life has been little investigated in the literature. Significant changes in the flavour, aroma and taste of refined and wholewheat bread have been reported by Jensen, Oestdal, Skibsted, Larsen, & Thybo (2011a). The sensory characteristics of refined bread and wholewheat bread during storage were studied by measuring volatile and non-volatile compounds and performing a descriptive sensory profiling (Jensen, Oestdal, Skibsted, Larsen, & Thybo, 2011a). Refined and wholewheat bread showed distinctive flavours, revealing two different sensory profiles (Jensen, Oestdal, Skibsted, Larsen, & Thybo, 2011a). Storage time affected 8 out of 13 of the tested attributes of refined bread, while all 13 attributes of wholewheat bread were significantly impacted by storage time (Jensen, Oestdal, Skibsted, Larsen, & Thybo, 2011a). The fresh wholewheat samples were characterized by higher concentrations of fermentation products; after one week of storage, dough and bran aroma were the predominant attributes, while breads stored up to 2-3 weeks were defined by rancid and fatty aromas, and a bitter taste (Jensen, Oestdal, Skibsted, Larsen, & Thybo, 2011a). The formation of off-flavours in bread could be related to the formation of secondary lipid oxidation products during storage together with a reduction in compounds from Maillard reactions (Jensen, Oestdal, Skibsted, Larsen, & Thybo, 2011a). Since UWFs are characterized by higher enzymatic activity and lipid and antioxidant contents than refined flours, the development of specific strategies for the control of oxidative reactions represents a key factor for improving bread storage (Doblado-Maldonado, Pike, Sweley, & Rose 2012).

3.4.2 Packaging of UWF breads

Several packaging strategies have been developed to preserve bread freshness. The main objectives of these methods are to prevent microbial spoilage and bread staling. Bread packaging has been studied on refined bread, while no techniques have been specifically developed to preserve UWF bread.

Therefore, here we discuss the packaging strategies that appear promising to us for extending the shelf life of UWF breads.

Packaging methods are classified as conventional and active packaging. The former includes traditional packaging methods, aimed at preserving the food from chemical, physical and biological damage without interacting with it. Conversely, active packaging is based on the interaction between the packaging material and the food matrix by absorbing or releasing specific substances (Melini, & Melini, 2018).

WG makes UWF particularly susceptible to lipid oxidation (Boukid, Folloni, Ranieri, & Vittadini, 2018), and the fibre component also impacts the product moisture during storage time (Hemdane et al., 2016). Hence, the critical aspects of UWF storage could be identified as rancidity phenomena and higher moisture retention, linked to microorganism spoilage.

Active Packaging with Antimicrobial Releasing Systems could be useful in preventing UWF bread spoilage. These methods release antimicrobial agents (organic acids, fungicides, alcohols and antibiotics) into the food surface, thus inhibiting or delaying microbial growth and spoilage (Melini, & Melini, 2018).

Active Packaging with oxygen absorbers could be even more interesting for UWF (Alhendi & Choudhary, 2013; Melini, & Melini, 2018). In fact, Nielsen, & Rios (2000) observed that oxygen absorbers combined with essential oils prevented microorganism spoilage. Furthermore, Latou, Mexis, Badeka, & Kontominas (2010), by combining oxygen absorbers with an alcohol emitter, and Tian, Decker, & Goddard (2012), using metal chelating carboxylic acids, reported effective prevention against microorganism spoilage and lipid peroxidation.

The innovative trend in Active Packaging includes the application of nanotechnology, a fusion of traditional packaging polymers with nanoparticles. These methods are able to extend a product’s shelf life, while reducing the addition of preservatives in the food formulation (Melini, & Melini, 2018). Being a “natural” approach, nanotechnology could be applied to UWF bread, since it could preserve the high nutritional value of the product. Silver nanoparticles included in polypropylene food containers were reported to keep bread fresher over 3 or 4 times longer and to reduce bacterial growth by 95% compared to conventional food containers (Bumbudsanpharoke et al., 2015). Moreover, nanoencapsulation applied to essential oils, which show potent antimicrobial and/or antioxidant properties, may represent another promising technique for UWF bread. This method protects the compound against chemical reactions and undesirable interaction with the food matrix (Melini, & Melini, 2018). Nanoencapsulated essential oils extend the shelf life and maintain the sensory properties of breads (Otoni, Pontes, Medeiros, & Soares, 2014; Gutiérrez, Batlle, Andújar, Sánchez, & Nerín, 2011; Souza, Goto, Mainardi, Coelho, & Tadini, 2013). However, nanoparticles are not inert materials: they may interact with food, its surroundings and negatively impact human health. Therefore, there is an urgent need to assess the risks of this innovative method (Alhendi & Choudhary 2013; Melini, & Melini, 2018).

4. The carbon footprint of UWF bread

Technological innovations sometimes have negative environmental effects, such as the emission of greenhouse gases (GHG) and waste as a result of manufacturing activities. The Carbon Footprint (CFP) is a useful tool to quantify GHG emission during the life cycle of a product/service, allowing an estimation of its environmental impact. The food industry, including food production, preservation and distribution, consumes a considerable amount of energy which contributes to total CO_2 emission (Roy et al., 2009). Furthermore, consumers in developed countries require safe foods of a high quality, produced with a minimal impact on the environment (Boer, 2002), showing that sustainability will soon become a primary factor in making food choices a part of food quality criteria (Andersson, Ohlsson, & Olsson, 1994; Pattara, Russo, Antrodicchia, & Cichelli, 2016).

In the literature several papers have analysed the CFP associated with the life cycle of wheat bread (Holderbeke, Sanjuán, Geerken, & Vooght, 2003; Braschkat, Patyk, Quirin, & Reinhardt, 2003; Rosing & Nielsen, 2003; Roy et al., 2009; Pattara, Russo, Antrodicchia, & Cichelli, 2016; Meisterling, Samaras, & Schweizer, 2009; Notarnicola, Tassielli, Renzulli, & Monforti, 2017; Laurence, Hartono, & Christiani, 2018). These studies have shown that the main hotspots in the bread supply chain are the agricultural phase, primarily due to the use of pesticides and fertilizers, followed by the baking, mainly performed with an electric source of energy. The consumption of bread, including refrigerated storage or toasting, has an important environmental impact too (Holderbeke, Sanjuán, Geerken, & Vooght, 2003; Rosing & Nielsen, 2003; Meisterling, Samaras, & Schweizer, 2009; Espinoza-Orias, Stichnothe, & Azapagic, 2011; Notarnicola, Tassielli, Renzulli, & Monforti, 2017; Laurence, Hartono, & Christiani, 2018). Conversely, the CFP associated with the phases of packaging and transport still deserve discussion (Roy et al., 2009).

To the best of the authors' knowledge, only one paper has considered flour composition as a variable for CFP estimation (Espinoza-Orias, Stichnothe, & Azapagic, 2011). This paper reported the hot spots in the life cycle of packaged sliced breads from refined, brown and wholewheat flours produced and consumed in the UK (Espinoza-Orias, Stichnothe, & Azapagic, 2011). The key findings showed that the CFP of bread ranges from 977 to 1244 g CO_2eq per loaf of bread (defined as 800 g), and that thick-sliced wholewheat bread packaged in plastic bags has the lowest CFP while medium-sliced refined bread in a paper bag has the highest. The degree of refinement of the flour used in the bread recipe made a significant environmental impact: the higher the milling extraction rate, the lower the CFP (Espinoza-Orias, Stichnothe, & Azapagic, 2011). This means that bread with higher degrees of refinement is more ecologically sustainable. However, the reported results could be attributed to UWF breads produced with raw materials not subjected to additional processing. On the other hand, the current literature does not evaluate the environmental impact associated with those techniques designed to increase the technological quality of UWF bread. In our opinion, this topic deserves deeper investigation, considering that environmental impact has becoming an essential quality criterion for a food product (Pattara, Russo, Antrodicchia, & Cichelli, 2016). Therefore, in evaluating the best processing techniques for breadmaking with UWF, computation of the CFP should be included as a quality requirement.

5. Conclusions

The present review reported the main techniques and technologies that have been specifically developed for the use of UWF in the breadmaking process. Although the consumption of UWF breads characterized the greatest part of human history, the introduction of the roller mill in the 19th century led to the use of refined flour with a better technological performance, longer shelf life and sensory quality largely appreciated by modern consumers. Hence, refined flour has become the standard in the development of the quality tests, bread formulation and processing methods applied in each phase of the breadmaking process.

In recent years, studies about the positive effects of wholegrain consumption have led to a renewed interest in the employment of UWF in breadmaking. However, although the presence of various amounts of wheat bran and/or WG enhances the flour's nutritional value, these supplementations significantly change its composition. As a result, a different raw material, that is, UWF, can be used as a substitute for refined flour in bread production. The following points summarize the main consequences that the re-introduction of UWF have brought:

i) The standard tests to predict the breadmaking attitude of flour have remained unchanged, often giving an improper evaluation of the potentiality of UWF (i.e., water absorption capacity of the flour).

ii) The main efforts to improve the quality of UWF bread have been focused on optimizing the bread formula with the inclusion of various improvers.

iii) Little research has been conducted on modifying the processing variables of the breadmaking phases (i.e., mixing, resting, leavening and baking); practically the same methods developed for refined flours are adopted for the production of UWF bread too.

In our opinion, the different composition of UWF requires specific adaptation of the quality tests, so that this may improve both the technological evaluation and the use of UWF in bread production. Furthermore, new processing methods specifically adapted for the chemical characteristics of UWF may require further investigation as strategies to both preserve the high nutritional value and increase the technological quality of the final products, hence promoting the consumption of healthy foods.

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3. Aim of thesis

The actual market demand is requiring healthy and functional foods, since the nutritional quality has become a critical factor driving consumers' choices (Schaffer-Lequart et al., 2017). However, since the chemical composition of unrefined wheat flours is different from refined flours, the adoption of the standard bread-making process results in low-quality doughs and breads (Hemdane et al., 2016; Boukid et al., 2018). The literature reported in the previous review article (article 1) showed that in order to face the issue of using unrefined flours for bread production, food technologists has mainly proposed the use of improvers, whereas the possibility of changing the processing conditions has been scarcely investigated (Parenti et al., 2020a). Consumers also demand for clean label formulas; hence, the actual literature studies do not seem to have met market requirements (Guerrini et al., 2019).

Therefore, the main focus of the Doctoral thesis was identified in the development of innovative techniques and technologies to improve dough properties and bread quality when using unrefined wheat flours in the bread-making process. In the present research, the adoption of a different approach when using unrefined wheat flours for bread production has been proposed. The standardization approach, largely used for refined wheat flours, is considered not appropriate for unrefined wheat flours, but an adaptation of the bread formula and the processing conditions as a function of the flour requirements was suggested, in order to improve the exploitation of unrefined wheat flours in the bread-making process.

The innovative bread-making techniques and technologies the Doctoral thesis intended to explore are the ones that mostly meet the consumers' demand for more natural and healthy breads. This approach was pursued during the overall research study: the technological innovations aimed to increase the product quality should also preserve the nutritional value of unrefined wheat flours. Following this approach, the minimal addition of natural improvers as well as the investigation of innovative processing conditions having the minimal impact on wheat flour constituents were investigated.

The possibility of producing unrefined breads with acceptable technological quality would have important implications. Bread is a staple food in many diets around the world, hence providing breads with better nutritional value would significantly affect the daily nutrient intake with positive health benefits on human beings such as the prevention of chronic-degenerative diseases.

4. Results and Discussion

4.1 Survey to bakers using unrefined and old wheat flours

4.1.1 Preliminary remark – article 2

The previous review article (article 1) reported the attempts that the scientific research has made to address the consumers' demand of unrefined wheat based products: the modification of the bread dough formula with the addition of several improvers resulted the most used approach. On the other hand, the operations of the bread-making process were almost left unchanged. This means that the possibility of adapting the process to the inherent characteristics of unrefined flours has not been much explored in the current literature (Parenti et al., 2020a).

Driven by these considerations, we realized that the bakers have probably been the first to face the consumers' demand for unrefined wheat breads. Therefore, we considered important for the thesis research topic to find out how the small bakeries and the baking industry have responded to the new consumers' requirements and how they faced the issue of substituting refined wheat flours with unrefined wheat flours in the bread-making process.

We also considered important for the bread-making with unrefined wheat flours the market trend observed during last years, which involved a particular interest in the flour genotype (Mefleh et al., 2019a; Boukid et al., 2020). Indeed, wheat was subjected to intense genetic selection during last century. Between 1930s and the late 1960s, the third Agricultural Revolution occurred, commonly known as the "Green Revolution". The Green Revolution is defined as a set of research and technological transfer initiatives spreading all over the World. In Italy this revolution was initiated by Nazareno Strampelli, who was among the first to systematically apply Mendel's laws to traits such as rust resistance, early flowering and maturity, and short straw. The direct consequence in Italy was that wheat production doubled. After the Second World War, some of the Strampelli's wheat varieties were used as parents in breeding programs in many countries, leading to the development of the high-yielding wheat varieties. Thereafter, during the 1960s, research focused on improving the storage protein quality (i.e. the gluten proteins), hence increasing the technological properties. The main result was the development of modern varieties generally defined as "high-yielding varieties" since they are characterised by a higher capacity of nitrogen-absorption which required the introduction of semi-dwarfing genes to reduce wheat lodging and the maturation cycle. The main features of modern wheat varieties include higher yield, a reduced susceptibility to disease and insects, an increased tolerance to environmental stresses, a homogeneous maturation and a higher gluten quantity/quality (to improve bread and pasta technological quality). These intensive genetic programs helped to increase wheat production and technological quality, but the replacement of ancient local breeds with modern varieties caused a concomitant decrease in the genetic variability of wheat genotypes (Dinu et al., 2108; Mefleh et al., 2019b). Furthermore, these breeding programs did not consider the characteristics of the bran and the germ components since these fractions were both eliminated during the milling process, hence they neither contributed to the flour technological performance nor to the sensory profile of the final product.

Although there is not an official definition of “old wheats”, this term generally refers to the wheat varieties that were selected before the intense genetic selection on wheat technological quality occurred in the 1960s (Dinu et al., 2018; Mefleh et al., 2019b). As a consequence, the old wheat category includes a wide range of wheat cultivars making it difficult to identify common characteristics. However, some scientific articles showed interesting results on some old wheat varieties which were reported to have a high nutritional value and potential health benefits (Leoncini et al., 2012; Dinelli et al., 2011; Sofi et al., 2010; Gotti et al., 2018; Sereni et al., 2017). Due to these positive features, there is an increasing consumers’ demand for products obtained from old wheat flours, although the most used varieties have shown poor technonological quality (Bassignana et al., 2015; Ghiselli et al., 2016; Cappelli et al., 2018; Farbo et al., 2020).

Following these considerations, we decided to conduct a survey to the Tuscan bakers (article 2) performing the bread-making process with unrefined wheats that belong to old wheat category. The scope of the study was to obtain an empirical state of the art about the techniques and technologies that the baking industry has developed to obtain both an acceptable technological and nutritional quality of breads from old unrefined wheat flours, hence addressing the new consumers’ demands.

4.1.2 Article 2

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The bread making process of ancient wheat: a semi-structured interview to bakers

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Abstract The importance of bread made from ancient wheat flours is currently increasing. In literature, several works examine the cultivation phase of ancient wheats, as well as their positive effects on human health. On a technological level, their bread-making process is hindered by the poor workability of these flours, but there are only few scientific studies aimed to enhance their technological performance. However, bakers developed several strategies to improve ancient wheat flour processability. We chose the semi-structured interview as instrument to investigate these strategies, evaluating them according to the existent literature. The study revealed that ancient wheats are usually stone milled, and processed as brown flours. Bread doughs are often prepared with flour blends, resulting from the cultivation of grain mixtures or obtained as flour mix.

The choice of slow mixers, an accurate monitoring of the final leavening phase and the use of sourdough, as well as the selection of flour blends have been proposed as solutions to partially improve the technological performance of ancient wheat flours. Finally, ancient wheat varieties are usually processed following several "good working practices" (i.e. use of non-refined flours, sourdough, organic cultivations) which probably play a role in enhancing their beneficial effects on human health.

Keywords
Triticum aestivum L.; ancient wheat varieties; baking; sourdough

Abbreviations
AWs, ancient wheats; AWVs, ancient wheat varieties; AWFs, ancient wheat flours

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1. Introduction

Consumed by billions of people, wheat (Triticum aestivum L.) is the major staple food in many diets, providing a large proportion of the daily energy intake, and it is currently cultivated worldwide. About 95% of the wheat produced is Triticum aestivum L., usually called “common”, “bread” or “soft” wheat (Dinu et al., 2018).

There is not a precise definition of AWVs, but generally this term refers to varieties cultivated before the intensive selection programs occurred during the “Green Revolution”. This intensive genetic selection led to the development of high-yielding modern varieties, characterized by higher capacity for nitrogen-absorption than other varieties, reduced susceptibility to disease and insects, an increased tolerance to environmental stresses, a homogeneous maturation, an improved storage protein quality and a higher gluten content. Whilst these breeding programs improved wheat yields and technological quality, a strong decrease of genetic variability occurred in modern varieties. As a consequence, AWVs are characterized by a broader genetic base than modern ones and, therefore, can be considered as a potential source of biodiversity (Dinu et al., 2018).

In recent years, there has been a re-discover of AWVs, probably due to the increased consumer attention to healthy and functional foods. Indeed, many scientific evidences showed the functional properties of AWVs (Di Silvestro et al., 2012). Moreover, studies on regular consumption of bread made with AWVs showed beneficial effects on human health: a reduction of metabolic risk factors, markers of both oxidative stress and inflammatory status (Sofi et al., 2013) and an improvement of lipidic, inflammatory and hemorheological parameters (Sofi et al., 2010).

Despite these health positive effects, the use of AWVs for bread production is hindered by some technological issues. Indeed, AWFs usually produce doughs characterized by low strength, high tenacity and low extensibility (Cappelli et al., 2018).

The lower technological properties of AWFs result in poor quality products if a standard process is applied. Therefore, the use of AWs for the bread making cannot rely on the standard process, but it requires appropriate techniques, specifically designed for these flours. Overcoming bread making issues could lead to a greater use of AWFs, promoting the consumption of products with enhanced nutritional value.

In literature, appropriate bread baking methods purposely developed for the different requirements of AWVs are currently lacking. On the other hand, in Tuscany, in the last decade, an increasing number of bakeries have started to produce bread from AWs, developing several strategies, protocols and techniques to improve bread quality. Tuscan bread made with AWFs have generally round or elongated shape, weight-size of 0.5-2 kg, fine-grained crumb and thick and crispy hazel crust.

The present study uses a qualitative interview to report the main techniques currently adopted in Tuscany for the bread making process with AWFs. The aim was to reveal the bread making methods currently implemented for AWFs and to find out the existing processing strategies that improve AWFs' workability. Qualitative methods, based on semi-structured interviews, were applied in order to gain a better understanding of the process from the baker's point of view.

2. Methods

2.1 Study design and interview method selection

Our study was carried out using qualitative semi-structured interviews following the systematic methodology described by Kaillo et al. (2016). First of all, we evaluated the appropriateness of the semi-structured interview as a rigorous data collection method in relation to our research question. The analysis of the literature revealed a lack of information about bread making techniques specifically designed for AWFs. Considering that AWFs have different characteristics compared to the modern flour blends commonly used in the baker industry, the standard bread making process became inappropriate and new processing strategies had to be designed. It seems very likely that the bread makers have been the first to face the different behaviour of AWFs during each phase of the process. Hence, in the absence of scientific studies on this issue, we decided to directly consult the experts in the field (i.e. bakers) in order to provide an empirical state of the art about the bread making techniques currently developed to improve AWFs workability. To gain this purpose, a qualitative analysis based on a semi-structured interview seemed to be the best method to obtain the bakers' expertise of the study phenomenon. In fact, this interview format consists of a set of predetermined open-ended questions, with other questions emerging from the dialogue between interviewer and interviewee. Therefore, using a semi-structure interview format could provide a deep insight of the research topic.

2.2 Sample selection

The sample was selected using the handpicked sampling method: we identified some reliable mills that milled AWs in Tuscany and used them as a reference point for bakers' selection. The chosen mills were 5: Molino Paciscopi (Montespertoli, Florence, Italy); Molino Val d'Orcia (Pienza, Siena, Italy); Molino Parri (Sinalunga, Siena, Italy); Molino Grifoni (Pagliereccio, Arezzo, Italy); Azienda agricola Floriddia (Peccioli, Pisa, Italy). They gave us addresses of well-known bakers that use AWF in the bread-making process. We selected the sample to be, to the best of our possibilities, representative of the AW bread production in Tuscany. We collected 20 interviews with bread makers; the number of participants was based on the saturation criterion: when no new themes emerged, we stopped acquiring the data (Marshall, 1996).

2.3 Interviews

A preliminary semi-structured interview guide was designed on the basis of key themes identified from issues prevalent in the research literature. The main themes were the following: characteristics of all the ingredients used in the recipe to prepare the bread dough, the accurate description of each phase of the bread making process: mixing, resting, leavening and baking. We also included, at the end of the interview, experts' opinions about the main problems linked to the use of AWFs for bread production. The interview framework was created as a list of questions, which helped the interviewer to direct conversation towards the research topic and to achieve the richest possible data. The semi-structured interview guide was then pilot tested in order to make some adjustments to the questions and to improve the quality of data collection. At first, we performed an "internal testing", which referred to the evaluation of the preliminary interview guide in collaboration with the investigators in the research team. In a second step we conducted both an "expert assessment" and a "field-testing" by exposing the preliminary interview to the critique of a reliable baker, who was also one of the potential study participants. This phase was particularly beneficial to our interview guide, because it simulated the real interview situation and it provided valuable guidance about the wording and arrangement of the questions.

Fig. 1 shows the final interview structure used to guide data collection throughout the whole survey. We decided to use the telephone as a medium to perform the interviews for its methodological strength in qualitative research (Cachia and Millward, 2011). All the interviews were performed by the same investigator. During the interviewing, the interviewer attempted not to take any leading position, but was a listener who gently directed the conversation to cover the main themes if necessary. Probing questions were asked to verify the right interpretations of answers. The duration of the interviews varied from 30 to 60 min. Data were noted down during the interviewing activity and immediately transcribed in order to accurately record the whole details expressed by the experts.

Ingredients
- Milling method
- Flour refinement degree
- Species and cv of ancient wheat grains
- Bread formula

Sourdough formula and management
- Sourdough formula
- Sourdough processing method

Bread making procedure
- Final mixing
- Resting
- Dividing and moulding
- Final leavening
- Baking

Final considerations
- Issues of using ancient grains for bread making
- Key steps of the baking process
- Main attributes of a quality bread

Fig. 1. Semi-structured interview schedule–topic guide.

2.4 Data analysis

Analysis of the qualitative data was performed according to the approach described by Elo and Kynga (2008) and Green et al. (2007). We identified the inductive content analysis as appropriate for our data, since we started collecting specific and individual data about bread making techniques with AWs, with the aim to achieve a general description of the processing methods.

The analysis process included four key steps: immersion in the data, coding, creating categories, and the identification of themes. Data analysis was done as follow. Immersion in the data is the first stage in the analysis process; it allows a detailed examination of what is said and lays the foundation for connecting disjoined elements into a clearer picture of the issue under investigation. The second step is coding, that is a process of examining and organizing information in each interview and the whole dataset. It forces the researcher to begin to make judgements and tag block of transcript. Categories represent the linking, a "good fit" between codes that share a relationship. The final step is the identification of themes, which is the litmus of a test study; it involves a description of a range of categories, and the interpretation of the issue under investigation (Green et al., 2007). To increase the credibility of this qualitative research, two co-authors independently analysed the same transcriptions and then compared their coded data. This triangulation strategy with multiple analysts helped reduce the potential bias resulting from a single person performing all of the coding and analysis (Patton, 2002). Both investigators undertook analysis, and reliability was enhanced by double coding a subset of transcripts and comparing inter-rater reliability.

3. Results and Discussion

As first result of data analysis, all the respondents underline the importance of making further technological improvements, and developing new strategies for the AWFs processing.

3.1 Ingredients

More than 50% of interviewees (i.e. 13) are used to buy flours at their trusted mill; almost 25% (i.e. 6) directly cultivated the grains that they transformed into flours at the mill. Four of them have sufficient amount of grain for their bread making requirements, while the other 2 have to buy some of the flours to support the baking production.

AWFs are generally stone milled: except one, all interviewees preferred this milling method than the roller mills. The common belief is that stone mills are better than roller ones on a nutritional level. However, literature shows that stone mills generate considerable heat due to friction, which could result in considerable damage to starch, protein, and unsaturated fatty acids in comparison with other milling techniques. Undoubtedly, there appears to be a marketing advantage using the term "stone ground" with consumers, as evidenced by the preponderance of unrefined wheat flour products making this claim in both retail and commercial markets. Thus, the interview highlights a discrepancy between the literature and the bakers' choice, which deserves deeper investigations. However, the milling phase is commonly depicted as a key step of the whole process; this is consistent with literature since the milling operation of unrefined flours appears to have a great impact both on flour composition and on technological properties, hence on the overall baking performance (Doblado-Maldonado et al., 2012).

Brown flour (i.e. type 2 according to the Italian classification) is the most common refinement degree, being chosen by the 50% of interviewees. The second prevalent trend (i.e. 7 bakers) is the contemporary use of flours characterized by different refinement degrees: brown flour, type 1 (according to the Italian classification) flour and whole meal flour. Unrefined flours show a higher nutritional value and several positive effects on human health, related to the protection from chronic diseases like cardiovascular disease, type 2 diabetes and various types of cancer, as well as an improved weight regulation (Ye et al., 2012). Despite the health positive effects, wheat bran addition in bread formulation results in a reduction of loaf volume, an increase in crumb firmness, a dark crumb appearance, a higher water dough absorption and a reduced fermentation tolerance (Boita et al., 2016). Specifically, the negative effects of the fibre components on dough technological properties was linked to the gluten dilution, affecting the dough gas-holding capacity; the disruption of the gluten matrix; and to the competition for water with other polymers, reducing the dough's viscoelastic properties (Boita et al., 2016). Hence, brown flours seem to represent the best compromise between nutritional benefits and technological issues: they show a richer nutritional profile than refined flours, since they contain the germ and some of the external bran components; moreover, the partial removal of the outer fibre fractions seems to improve their processability as compared to that of whole meal flours.

Figure 2 resumes the AWs selected for bread production. Triticum aestivum L., is the wheat specie preferred by all participants. Moreover, in addition to common wheat, about 50% of the bakers (i.e. 11) also use Triticum durum. Two other species are more rarely selected, i.e. Triticum monococcum and Triticum dicoccum.

More than 50% of participants (i.e. 13) do not choose pure wheat varieties of Triticum aestivum L., but they use grain mixtures, directly obtained from the cultivation of different wheat varieties (i.e. wheat mixture). Flour mixtures can also be realized after the milling process, by blending pure flour varieties (i.e. flour blends). Figure 2 schematizes both trends. The two prevalent wheat mixtures belong to different agronomical choice: one is a mixture of Triticum aestivum L. and Triticum durum varieties, while the other includes only Triticum aestivum L. varieties. The cultivation of ancient grain mixtures is reported to provide higher yields, lower yield variability, and better technological properties of the flours obtained. Indeed, the mixed cultivation of different varieties of a crop species in varietal seed mixtures is known in literature as a low-tech method to increase and stabilize cereal yields and to reduce the dependence on pesticides. Moreover, the potentials of seed mixtures of wheat and barley to provide increased grain yields support the hypothesis that positive mixing effects may derive from beneficial interactions between the component varieties (Kiær et al., 2009).

Ten out of 20 respondents use flour blends (realized after the milling) instead of grain mixtures. Three bakers use a mix of different cultivars of Triticum aestivum L., while 6 a mix of flours from Triticum aestivum L. and Triticum durum. Blending the two different wheat species seems to improve the baking performance. Actually, this practice was reported to enhance the bread-making quality (Bakhshi et al., 1989). Moreover, Torbica et al. (2011) found out durum wheat flour as an improvement agent in bread making with common wheat flour having damaged protein structure. Blending activity for AWFs has a different purpose compared to conventional flours: it does not aim to meet functional quality standards for the product destination use, but it seems to improve the technological performance, as if a synergic effect among the different components of AWVs occurs. The latter issue is poorly described in literature; hence, studies aimed to understand the mechanisms underlying these apparent improvements are required to identify suitable flour blends for AWFs.

As Triticum durum varieties the most common choice is Senatore Cappelli (i.e. 8 interviewees).

Finally, about the 25% of bakers use pure varieties of Triticum aestivum L.. The most popular one is Verna (i.e. 6 bakers); the remaining varieties are utilized less frequently and in a comparable way.

The second most abundant ingredient of the bread formula is water. The common trend (i.e. 14) is to add water amount in the range of 55-75% (w/w flour weight). Only 2 use higher quantity of water (i.e. 77% and 80-85%), and 4 lower amounts, between 50% and 30% (i.e. 2 bakers add 50%, one 45% e the last one 40%). A large variability of the water amount added to the bread dough arose; however, a general trend in preparing highly hydrated doughs can be observed. Indeed, water content acts as a swelling agent, which is able to reduce the dough stiffness. Water plays a double role in the baking process: firstly, mixed with flour it allows the development of a visco-elastic dough and secondly, after baking, there is more or less water remaining in the product, which will affect the final product texture (Zhou et al., 2014). Hence, we can assume that developing high hydrated doughs will provide an improvement of the rheological properties. Specifically, a better balance between the alveographic parameters tenacity (P) and extensibility (L) could be achieved, by decreasing their ratio (P/L), that is usually higher than the optimal reference range (Cappelli et al., 2018). This could provide an improvement of the overall baking performance with AWFs.

As leavening agent, the sourdough appeared to be the most popular choice (only one instead of sourdough uses the sponge dough method). Sourdough bread is prepared from a mixture of flour and water that is fermented with typical yeasts in symbiosis with lactic acid bacteria (LAB), generally in ratio 1:100. Doughs made with sourdough become softer and less elastic, and breads show improved structure and flavour. Many inherent properties of sourdough rely on the metabolic activities of its resident lactic acid bacteria (LAB) during their fermentation: lactic fermentation, proteolysis, and synthesis of volatile compounds, anti-mould, and anti-ropiness production. Moreover, sourdough activities can improve the mouthfeel and palatability of whole meal bread without removing any nutritionally important components (Chavan and Chavan, 2011).

Considering the amount of sourdough added in the bread formula (Tab.1), 16 respondents use between the 10 and 30% (w/w flour weight) of sourdough in the final dough, while the other bakers add higher quantities.

Additional ingredients are in some cases used; although in Tuscany bread is traditionally prepared without salt, about 25% of participants (i.e. 6) reported salt addiction in the bread formula (in low amount, always less than 1% w/w flour weight) as a way to improve the technological properties of doughs. While excess salt use is problematic from a nutritional point of view, it has been shown to positively influence every stage of the bread production. Salt effects of most relevance for improving the poor technological quality of AWFs, seemed to be the promotion of a better gluten-structure development during the mixing step and the formation of a fine elastic crumb during baking (Belz et al., 2012).

The use of improvement agents for bread production is out of practice (i.e. 19 bakers do not add improvement agents). This trend is in accordance with the bakers' common purpose: the exploitation of AWFs to obtain high nutritional products, avoiding every kind of supplements.

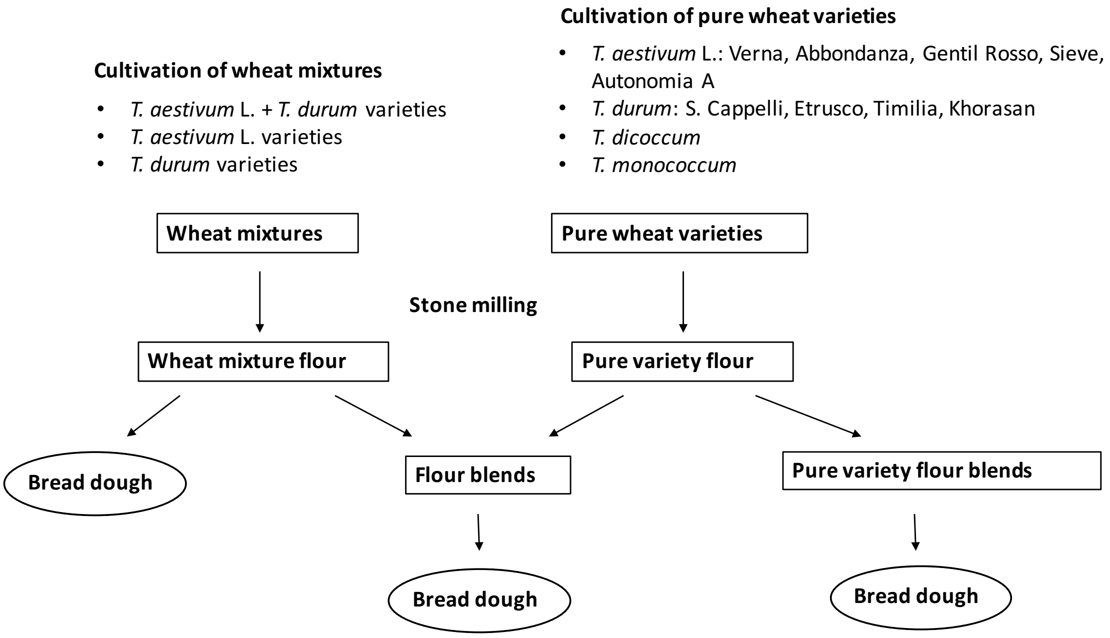

Fig. 2. The main AWs and flours selected for bread production. “Wheat mixtures” means AWs directly cultivated as grain mixtures; “flour blends” means mix of pure variety flours with each other or with wheat mixture flours realized after the milling process for bread baking.

3.2 Sourdough formulation and management

Sourdough is made with 2 ingredients: flour and water. The most common choice is to use the same AWF of the bread recipe; only 3 bakers prepare the sourdough adding modern wheat flours to facilitate the leavening phase. In literature is reported that the type of substrate, mainly flour, used for sourdough fermentation significantly influences the sourdough properties. Specifically, the bran fraction contains more minerals and micronutrients that are important for the growth of LAB (Chavan and Chavan, 2011).

Two different type of sourdough are used: 11 bakers prepare traditional sourdough (i.e. type 1 according to Chavan and Chavan, 2011), restarted by using a part of the previous fermentation and generally composed by firm dough with low water content (8 bakers add 40-55% w /w flour weight, while 2 use even less quantities 35% and 25%, w /w flour weight); 9 bakers create a high hydrated sourdough making a liquid suspension of flour in water (adding as much water as flour, i.e. 100%, w/w flour weight), which contains adapted strains to start fermentation (i.e. type 2 according to Chavan and Chavan, 2011). This choice significantly influences sourdough flavor profile; the firmer the sourdough, the more acetic acid is produced and the less lactic acid (Chavan and Chavan, 2011). Moreover, it also affects the dough acidification rate (Spicher and Stephan, 1999).

Due to its microbial life, the sourdough is metabolically active and its microflora is generally preserved at low temperatures, i.e. 4°C (i.e. only 4 preferred higher temperatures). To restart the sourdough activity before using it as leavening agent, a certain number of subsequent addition of flour and water followed by sourdough fermentation (i.e. propagation step), are usually carried out. In Tab. 1 the sourdough management and its frequency of usage are outlined. All bakers perform the first propagation step at room temperature and room moisture; only one uses low temperature during this step, T=4°C. The duration of this phase is extremely variable: times ranging from 1.5 to 8 h are selected by 13 bakers, while longer times (i.e. 12-24 h) are preferred by 6 bakers. Among the latter, 4 prepare liquid sourdoughs and the other 2 a traditional sourdough. Hence, liquid sourdough appears to need a longer propagation step as compared to the traditional one.

The large variability of the first propagation step could be associated to the complexity of sourdoughs as biological ecosystems, being characterized by different microbial composition. The dough hydration level and the flour compounds, the leavening temperature, and the sourdough storage temperature strongly affect the number and the type of yeast and LAB species found in doughs (Chavan and Chavan, 2011).

After the first propagation step, 11 bakers use the fermented sourdough as leaving agent for the bread dough preparation; all these participants perform a daily sourdough revitalization. Hence, performing just one propagation step appears to be the main trend for a daily sourdough management.

Conversely, a second propagation phase is carried out by 8 interviewees: environmental moisture and temperature are used by 7 respondents, while only 1 works at 4 °C. A strong variability characterizes also the duration of the second propagation step, since it ranges from 4 to 12 h. Looking at the frequency of sourdough utilization, we can observe that the great majority (i.e. 6 bakers among 8) use it once or twice a week (Tab. 1). Therefore, the number of the sourdough propagation steps is very likely dependent on the sourdough activity: a longer sourdough storage needs longer fermentation times to reactivate microorganism growth.

A third step is performed only by 3 participants (Tab. 1). It is carried out at room temperature and room moisture except for one (i.e. 4°C) and the duration of the step ranges from 3 to 8 h.

The same relationship between the frequency of sourdough utilization and the number of propagation steps cannot be observed for this last propagation stage, since 2 of the 3 bakers that perform it use the sourdough every day. Hence, this third step does not seem related to microorganism activity, but it is probably a working choice driven by other factors.

Table 1. Summary of the baking procedures from the 20 interviews. Sourdough management: % of sourdough amount in the bread formulation (% w/w$_f$ sourdough weight/flour weight); certain number (1, 2, 3) and duration (h) of sourdough propagation step/s; frequency (Freq.) of sourdough usage a week. Mixing step: mixer type, ingredient addition into the mixer (all ingredients added at the beginning or progressive addition of flour/water), total final mixing time (min). Resting time (h). Final leavening time (h). Baking step: oven type, temperature (T) profile (start temperature, T$_{st}$; final temperature T$_{fin}$); baking times of bread loaves <1kg and >1kg.

	Sourdough					Final mixing			Resting (h)	Final Leavening (h)	Baking				
n	(%) w/w$_f$	Propagation step (h)			Freq.	Mixer	Addition during mixing	t (min)			Oven	T profile		t (h) <1kg	t (h) ≥1kg
		1st	2nd	3rd								T$_{st}$ (°C)	T$_{fin}$ (°C)		
1	10-30	1.5-8	–	–	daily	fork	flour	10-20	–	1-2	gas	250	250	0.7	1
2	10-30	16	6	3	1/week	spiral	all at beginning	10-20	–	1-2	wood	220	200	–	0.5-1
3	10-30	1.5-8	–	–	daily	fork	flour	10-20	–	1-2	heat-cycle	250	250	0.5-0.7	1
4	10-30	1.5-8	4	–	1/week	by hand	flour	10-20	1-2	1-3	wood	300	280	–	1-1.25
5	10-30	1.5-8	–	–	daily	twin arm	water	10-20	<1	>4	wood	300-340	↓	–	1-2
6	10-30	1.5-8	–	–	daily	twin arm	water	10-20	<1	1-3	gas	220	190	0.5-1	–
7	30-40	1.5-8	–	–	daily	twin arm	all at beginning	10-20	1-2	1-3	wood	230	↓	0.8	–
8	10-30	1.5-8	0.5	–	daily	spiral/twin arm	water	10-20	1-2	1-3	electric	240-250	240-250	0.7	1
9	10-30	1.5-8	12	–	1/week	spiral	flour	10-20	12	1-3	wood	280	↓	0.7-0.75	1
10	30-40	1.5-8	7	–	2/week	spiral	water	<10	1-2	1	heat-cycle	240	215	0.8	–
11	10-30	14	–	–	daily	fork	all at beginning	10-20	<1	>4	wood	240	200	1	1.3
12	10-30	1.5-8	4-6	4-6	daily	twin arm	all at beginning	30	<1	1-2	wood	310	280	–	1
13	–	–	–	–	–	spiral	all at beginning	10-20	<1	>4	gas	220	220	0.7	1
14	10-30	24	–	–	daily	fork	water	10-20	–	1-3	wood	240	180-190	0.8	1.5
15	10-30	12	–	–	daily	by hand	water	30	–	1-3	wood	220	↓	–	1
16	80	12	–	–	daily	spiral	all at beginning	10-20	1-2	1-2	wood	220	↓	0.4	–
17	10-30	1.5-8	–	–	daily	twin arm	all at beginning	10-20	–	1-2	heat-cycle	220-230	200-210	0.75-0.8	–
18	10-30	1.5-8	4-8	4-8	daily	by hand	all at beginning	45	1-2	1	wood	300	↓	0.75-1	–
19	10-30	1.5-8	12	–	2/week	spiral	flour	10-20	<1	1-3	electric	210	180-190	–	1
20	10-30	12	–	–	3/week	twin arm	all at beginning	<10	1-2	1-3	wood	280-300	140	–	1

3.3 Final mixing step

Mixing is one of the most important step of the entire baking process, since most of the characteristics of the final product are determined directly or indirectly during this phase (Zhou et al., 2014).

In Tab. 1 the main features of the mixing step are shown. The most popular machines are the spiral mixer (i.e. 7 bakers) and the twin arm mixer (i.e. 6 bakers), with one baker having both spiral and twin arm mixers. Thus, there is not agreement in the choice of mixer and bakers adopt very different strategies. In fact, twin arm mixers produce a better dough aeration, providing a higher volume increase and oxygenation of the dough, and consequently higher volume of the final product. Conversely, the spiral mixer generates lower gas occlusion, and higher temperature increase during the mixing phase (i.e. 9-10°C vs 4-6°C) (Quaglia, 1984). The role of the oxygen within the dough is linked to the proper gluten matrix development: under the kneading action, the original unorganized disulphide bounds of gluten are broken by reduction and new ones are formed by oxygenation. This oxidation strengthens the dough and locks the new "structure" in place (Zhou et al., 2014).

Concerning the beginning of the mixing process, we asked about the order of ingredients' addition into the mixer. Nine interviewees put all the ingredients together in the kneader at the same time, while 11 follow a precise order (Tab. 1). Among the latter, two different working methods arose: a gradual addition of the water amount (i.e. 6) or a gradual addition of the flour amount (i.e. 5). Both these techniques aimed to achieve a progressive flour hydration during the mixing step, by dosing water or flour addition; bakers report that this technique provides a higher water absorption and a better dough development.

With regard to the total mixing period, short times (i.e. 10-20 min) are generally preferred (i.e. 15 bakers). The other bakers are split into two groups: 3 of them select longer mixing times, between 30 and 45 min, but among them, 2 bakers do not use a mixer, since they work the dough by hand; hence, longer times are probably linked to this different mixing method. On the contrary, the other 2 participants use shorter mixing times (i.e. <10 min). Choosing short times of the mixing phase seems to be essential to face the poor stability of AWFs, that cannot support longer mixing period without losing dough quality. Despite the environmental conditions during mixing are not controlled, an accurate monitoring of the dough temperature arises from the interview. Dough is kept around 24°C-26°C by adjusting the water temperature. This result is consistent with literature; it is well known that during mixing, the temperature of the developing dough begins to rise as a direct consequence of energy being transferred to the dough. The adjustment of ingredient temperatures, most notably the temperature of the water, produces dough with a consistent final dough temperature for uniform processing after mixing and optimises the final product quality (Cauvain and Young., 2007).

3.4 Dough resting and piecing

Once the mixing step is completed, 15 out of 20 respondents give to dough a resting period (Tab. 1): 1-2 h is the most frequent choice (i.e. 8), followed by shorter times, <1h (i.e. 6); the process occurs at room temperature and room moisture almost for all, hence, the resting period can vary in relation to the seasonal changes. During long resting times, care should be taken to prevent the dough skinning; all the interviewees report to accurately cover the doughs in order to preserve their original moisture. Several phenomena occur during the dough resting period. Yeasts start to generate carbon dioxide gas, increasing the bubble size within the dough and thus affecting the final bread cell structure. The extent of the activity depends on resting time and dough temperature. Furthermore, resting time can enhance the fermentation process, allowing a "natural" dough conditioning, thus requiring lower levels of reducing agents and other dough improvers to be used (Cauvain and Young., 2007).

Moreover, nearly the 50% of participants observed that during the resting period a better development of the gluten matrix could be obtained by folding the dough many times on itself.

The next phase of the bread making process is the dough make up which includes both the dividing step, that consists of cutting the dough (by hand or mechanically) into loaf size pieces and the moulding step, which consists of the dough pieces moulded into the desired shape. The major part of the bakers uses the dividing machine, while the moulding process is commonly carried out by hand in order to preserve the dough structure. Moulding step can notably affect the dough development; in fact, the gluten matrix acquires its final orientation under the moulding mechanical work (Quaglia, 1984). The most prevalent bread sizes are those of 0.5, 1 and 2 kg, but the survey also reveals a great demand of little sizes, less than 1 kg (i.e. 0.3, 0.5, 0.6 kg), since a great part of the consumers are used to buy fresh bread pieces every day.

3.5 Final leavening step

Leavening is one of the most important steps of bread making after mixing. The use of natural leavening may result in variation of the quality of the fermented dough due to variable types and amounts of microorganisms in the system. Yeast generates carbon dioxide gas, resulting in an increase in dough volume, and hetero-fermentative LAB yields lactic acid (which decreases pH), acetic acid (which acts as an antifungal agent), exo-polysaccharides (which can act as a gut health promoter), and volatile compounds (which act as a flavouring agent). In addition, protein is degraded through proteolysis to amino acids, promoting microorganism growth and the development of Maillard flavour compounds (Zhou et al., 2014).

All participants except 2 leave the doughs leavening without any containers; they state that bread doughs have to maintain their structures and shapes on their own. The 2 remaining bakers prepare highly hydrated doughs, and have to put dough pieces into moulds, since they are not able to develop a proper structure on their own. All bakers perform the leavening step at room temperature and room moisture. In Tab. 1 the duration of the leavening step is shown. The most common trend (i.e. 16), independently of the presence of the resting step, is to carry out a leavening phase of about 1-3 h, depending on the external conditions; the other 4 bakers select longer leavening times (i.e. >4 h). Moreover, the duration of the total leavening step changes with the environmental conditions, following the seasonal temperature trend throughout the year; longer times are necessary in cooler seasons while shorter times are appropriate in warmer conditions. The large variability of sourdoughs requires an accurate control of the leavening step. In fact, different microorganism-substrate interactions could be established in the leavening dough. As discussed below, bakers are used to manage this high variability of the leavening performance by keeping high the sourdough viability.

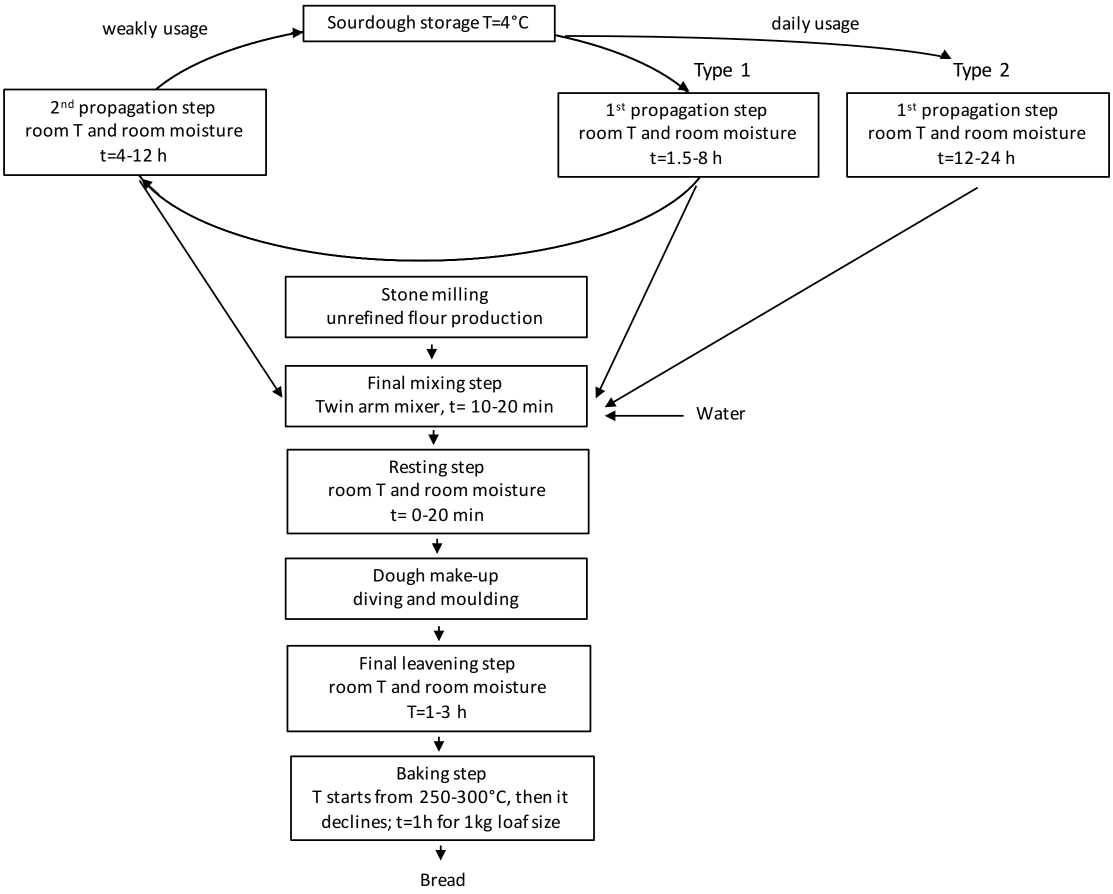

Fig. 3. The bread making process with AWFs in Tuscany.

3.6 Baking step

Tab. 1 shows the results of the baking step. More than the half part of the interviewees (i.e. 12) use a wood oven for baking. Other oven typologies used are: heat-cycle oven, gas oven, electric oven. With regard to the initial temperature, half of the wood oven users choose very high values, around 300°C, while all the others use values between 200°C and 250°C. During the baking phase, temperature naturally decrease in wood ovens; with the other oven typologies, 50% of bakers create a temperature drop, while the remaining bake in a steady condition. Therefore, regardless the different oven types, data reveals a common trend in the temperature profile management during the baking process: bread doughs are generally baked under a decreasing heat condition. Temperature profile markedly impacts product quality since it affects enzymatic reaction, volume expansion, starch gelatinization, protein denaturation, non-enzymatic browning reactions, and water migration (Zhou et al., 2014). Furthermore, it has been reported that the use of different baking temperatures affects the crust-crumb ratio of high-moisture products (Ahrne et al., 2007). High heat transfer limits the extent of the oven-rise because the gases do not have enough time to complete their potential for expanding cells. On the other hand, long baking times tend to decrease crumb softness (Cauvain and Young, 2007). The common use of higher temperatures at the beginning of the baking phase followed by a temperature decrease is probably linked to the necessity of obtaining a rapid crust development in order to create a thick crust and a soft crumb, both elements that characterized Tuscan bread quality. Moreover, it could be a strategy to improve the final product quality. In fact, the higher heat at the beginning of the phase can probably lock the loaf leavened structure without allowing any further expansion of its inner gas, which could not be retained by the weak AWFs. Hence, the volume growth obtained during the leavening step could be preserved.

Humidity level in the oven represents another key factor during the baking process. Wood ovens naturally provide a moisture release, hence, no additional moisture has to be added during baking. Tab. 1 shows that 3 out of the 9 bakers who do not use wood ovens are used to add moisture in the baking chamber, specifically in the first phase of the baking process in order to improve the final product quality. Indeed, steam injection delays the evaporation of water at the dough surfaces by condensation of water from the oven atmosphere onto the dough surface. Condensation proceeds as long as the crust temperature is below the dew point temperature of the oven atmosphere, which occurs during the first minute of baking (Zhou et al., 2014). This phenomenon produces a barrier on the dough surface against the release of carbon dioxide, resulting in a higher bread volume development and in a thinner, softer and lighter crust (Quaglia, 1984). When baked in a dry atmosphere, bakery products have a dull surface appearance and a rather harsh colour (Zhou et al., 2014).

The time taken to achieve a satisfactory baked product depends to a considerable extent upon the properties of the product itself, its surface/mass ratio, and its weight (Quaglia, 1984). The duration of the baking phase reported is close to 1 hour, or a little over, for bread doughs between 1 and 2kg, while it is around 30-40 min for smaller dough pieces. These data are consistent with literature; 30 min for 350 g dough with oven temperatures of 200 °C or 235-275 °C; and 35 min for 600 g dough at 225 °C (Zhou et al., 2014).

Figure 3 outlines the bread making process with AWFs in Tuscany resulting from our interview study. Furthermore, the interview allows to highlight the critical points in the bread making process as perceived by bakers. Figure 4 resumes the critical points and the number of bakers consider each of them important. We can observe that the most important working procedures resulted: the selection of short mixing times (i.e. 13); the identification of the proper leavening time (i.e. 9); the use of flour mixtures/blends (i.e. 7); the proper flour hydration (i.e. 6); the sourdough viability (i.e. 5).

Finally, before drawing conclusions, it is important to point out on some of the study limitations. First of all, results are obtained from a sample of 20 Tuscan bakers. The saturation criterion was adopted by the authors, to limit the drawback due to the number of respondents. However, more detailed information may arise from a bigger sample. The baking process here reported is intrinsically linked to the Italian and, more specifically, Tuscan tradition of bread making. Hence, in other parts of the world, different practices and techniques could be found by a similar interview.

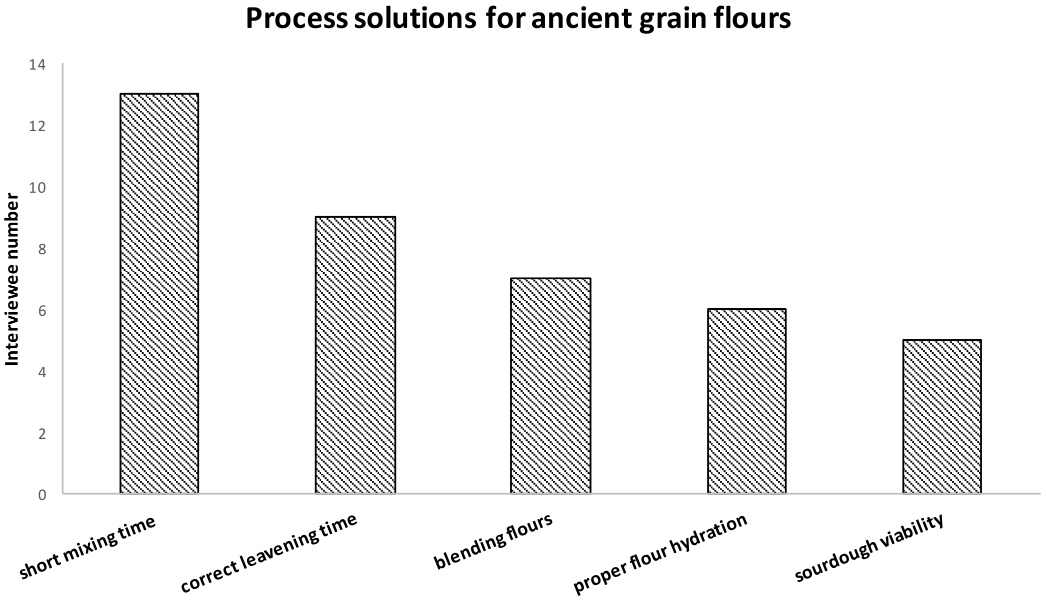

Fig. 4. The main critical points of the bread making process highlighted by the survey: short mixing time, correct leavening time, blending flours for bread formulation, maintenance of the sourdough viability.

4. Conclusions

In the literature many papers focus on the evaluation of AWF genetic, agronomic and nutritional characteristics, while few studies focus on AWFs' bread making process. On the other hand, bakers develop several strategies to improve the AW final bread quality. Hence, we performed a survey to find out the techniques currently adopted by Tuscan bakers.

It has been extensively reported that these flours positively affect human health as well as they yield environmental benefits. However, the adoption of several "good working practices" during the whole production process enhances the AWFs positive effects on environment and health. In fact, AWFs are baked with the purpose to preserve their valuable nutritional value during each phase of the transformation process, from farming to flour exploitation during baking (i.e. organic cultivation, use of non-refined flours, use of sourdough). All these choices provide environmental and nutritional positive effects, regardless if flours are ancient or modern. However, on a technological level, adopting these good practices sometimes results in a worsening of the already poor AW grain workability.

The main "good working practices" revealed by the survey involve a cultivation step under organic farming and a milling phase performed with stone-mills, which results in unrefined flours as products. Therefore, AWFs contain significant amounts of fibre and germ in their composition, which negatively affect the dough workability but improves the bread nutritional values. With regard to the bread formula, the interview discloses a common trend to realize grain mixtures or flour blends with the aim to achieve both an agronomic advantage and an improvement of the flour workability.

Concerning the baking process, our results reported some common processing methods that appear to increase AWF workability as well as the final product quality. These baking methods include: the use of the sourdough as a leavening agent, the optimization of the sourdough activity, short mixing time to optimize the dough development, a gradual flour hydration throughout the mixing, a resting period after the mixing phase (around 1 h), a long leavening step (around 3 h), and a baking step performed in wood ovens, with high temperature at the beginning followed by a heat decline.

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4.2 The effect of flour pre-gelatinization

4.2.1 Preliminary remark – Article 3

The survey (article 2) disclosed the main practices that bakers have developed to use old and unrefined wheat flours in the bread-making process. It is interesting to note that bakers' response to this issue was the opposite compared to that of the scientific research. Indeed, among bakers the prevalent trend was to use the basic ingredients for the bread dough formula (i.e. flour, water, and yeast) without the inclusion of improvers and to introduce some processing strategies that seem to improve the bread-making performance of unrefined and old wheat flours. Among these strategies, those most commonly adopted included the use of short kneading time, the identification of the correct leavening time, the use of different flour varieties in the bread dough, the inclusion of high water amounts and the maintaining of the sourdough viability. However, all bakers expressed the need to find innovative techniques to improve the technological performance of old and unrefined wheat flours (Guerrini et al., 2019).

On the other hand, the review (article 1) highlighted that the main strategies developed in the scientific research to improve the technological performance of unrefined wheat flours involved the addition of bread improvers (Parenti et al., 2020a).

The development of these two opposite answers to address the same issue showed that there has been a gap between scientific research and baking requirements. Indeed, the literature results showed that improvers are useful in improving the bread-making performance of unrefined flours (Parenti et al., 2020a), but the fact that bakers decide to not use this solution is a clear indication that consumers are also demanding clean-label formulas (Guerrini et al., 2019).

Considering the results of these two studies, we decided to focus the thesis research on the evaluation of natural bread improvers, which could have a positive technological effect while preserving the valuable nutritional value of unrefined wheat breads. The main scope of the research was to find technological solutions able to both address consumers' requirements of technological and nutritional quality.

In the following study (article 3) we decided to investigate the effect of a natural bread improver coming from a Japanese technique. The improver consists of the same basic ingredients of the bread formula but they are partially processed to achieve a different physical form compared to the native form. Before the bread-making, part of the bread flour and water are subjected to a gelatinization process and cooled to room temperature to form pre-gelatinized flour. This improver prepared from different food sources has recently shown positive effects on bread texture and shelf life (Carrillo-Navas et al., 2016; Fu et al., 2016; Kim et al., 2017; Zettel et al., 2014), but the effects on the bread-making performance of unrefined wheat flours have not been investigated.

4.2.2 Article 3

Parenti, O., Guerrini, L., Canuti, V., Angeloni, G., Masella, P., & Zanoni, B. (2019). The effect of the addition of gelatinized flour on dough rheology and quality of bread made from brown wheat flour.

LWT - Food Science and Technology, 106, 240–246.

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The effect of the addition of gelatinized flour on dough rheology and quality of bread made from brown wheat flour

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Abstract Although brown wheat flours are healthier than refined ones, baking quality is poor. To improve the workability and quality of brown wheat flour, we tested the addition of gelatinized flour during the production of salt-free bread. Dough rheology and bread quality were investigated in two trials. The first tested the addition of three levels of water and two levels of gelatinized brown flour. Brown flour gel addition significantly affected dough rheology and bread quality. Doughs made with gel required more water. Furthermore, interactions statistically significant between gelatinized brown flour and water content were found for bread volume and crumb hardness. The second trial tested the effects of gelatinized brown flour addition in doughs prepared with optimal water content (gelatinized flour samples required more water to reach optimum levels). Dough rheology was improved with the use of gelatinized brown flour; bread samples had significantly higher volume and lower hardness and chewiness. The addition of gelatinized brown flour may represent a good strategy to improve the baking performance of brown wheat flours, notably dough rheology and bread quality. The technique does not require the addition of new ingredients and preserves the high nutritional value of brown flour.

Keywords
salt-free bread, starch gelatinization, Farinograph, Alveograph, texture analysis

LWT - Food Science and Technology (2019) 106, 240-246

1. Introduction

The wheat kernel consists of three main parts (the embryo or germ, the outer coating or bran, and the endosperm), which are anatomically and chemically different from each other (Khalid, Ohm & Simsek, 2017). Like other baked goods, the majority of bread is made from refined wheat flour. This lacks the outer layers that are rich in important nutritional elements that are beneficial to human health, such as dietary fiber, fat, antioxidant nutrients, minerals, vitamins, lignans and phenolic compounds (Khalid, Ohm & Simsek, 2017). According to Zhou, Therdthai, & Hui, (2012), refined or white flour usually corresponds to 75% w/w of the whole grain, while two other categories can be distinguished based on the extraction rate and refinement properties: wholemeal and brown flour. The former corresponds to approximately 100% extraction yield (ash content = 1.3–1.7 g/100 g dm) and is made from the whole grain with nothing added or taken away. Brown flour usually contains about the 85% of the original grain (maximum ash content = 0.95 g/100 g dm) as some of the bran and germ is removed.

Epidemiological studies show that including whole grains and cereal fiber in the diet protects from chronic diseases such as cardiovascular disease, type 2 diabetes and various types of cancer; it also may improve weight regulation (Ye et al., 2012).

Despite the health benefits of whole grain cereal products, consumption remains much lower than that of refined products in several countries (Rosa-Sibakov, Poutanen & Micard, 2015). The barriers to increasing consumption of unrefined grain products include consumer taste preferences, the inability to identify unrefined grain foods, the difficulty of substituting unrefined grains for existing ingredients in meals, availability, and price (Kuznesof et al., 2012). Furthermore, the storage of wholemeal and brown flour remains problematic; shelf life is shorter than that of white flour due to lipid and lipase degradation (Doblado-Maldonado et al., 2012). Moreover, the bran in these flours has a negative impact on the viscoelastic properties of dough, and bread made from unrefined wheat flour may have low loaf volume, a dense crumb structure, and a grainy, nutty, and bitter flavor (Zhang & Moore, 1997). The literature has linked several of these phenomena to the poor technological characteristics of unrefined flour. Scanning electron microscope images of whole wheat bread showed that bran components disrupt the gluten matrix (Gan et al., 1989), reducing the ability of gluten to maintain the loaf structure during fermentation and baking. Rosell et al., (2010) found that fibers compete for water with other polymers, decreasing the dough's viscoelastic properties and weakening them.

Given the high nutritional value of unrefined grain, various studies have sought to optimize quality parameters of whole wheat and brown bread (Hung, Maeda & Morita, 2007). Modified starches have been developed to limit some of the undesirable properties of native starches (Abbas, Khalil & Meor Hussin, 2010). Physical, chemical, and enzymatic treatments have been applied in order to obtain a huge range of starch applications, notably in the food industry (Abbas, Khalil & Meor Hussin, 2010). Starch is the major component in breadmaking, and plays an important role in the texture and quality of both the dough and bread (Abbas, Khalil & Meor Hussin, 2010).

Pre-gelatinization is a simple, physical way to modify starch. Heating starch to gelatinization temperature in the presence of a sufficient amount of water causes an irreversible molecular change. Its semi-crystalline structure transitions to an amorphous state, namely starch paste or gel (Goesaert et al., 2005). Starch gels from different sources may have an important role to play in improving dough and bread characteristics (Zettel et al. 2014; Kim, Kwak & Jeong, 2017; Fu et al., 2016; Carrillo-Navas et al., 2016). Starch properties have been related to loaf volume and, in particular, the pre-gelatinization temperature (Sandstedt, 1961).

The “Yukone”, “Yudane” or “Tangzhong” (water roux) method is a Japanese breadmaking technique that produces bread with a soft and sticky texture, and a high tolerance to staling (Naito et al., 2005 Kim, Kwak & Jeong, 2017, Yamauchi et al., 2014). A part of the wheat flour (usually 5–10% of the total flour mass) is mixed with water at > 60°C to trigger starch pre-gelatinization; the mixture is cooled to room temperature, then it is added to the other ingredients to obtain the dough (Naito et al., 2005). The literature suggests that the slow staling, low hardness and high cohesiveness of Yudane breads is mainly due to an increase in swollen starch, which, in turn, has been related to doughs with both higher water absorption and amylase enzymatic phenomena (Yamauchi et al., 2014).

This study investigated the effects of gelatinized brown flour (GBF) from wheat, prepared using the water roux method on doughs and breads. The aim was to improve dough processing and bread characteristics, thereby promoting the consumption of healthier foods and the intake of dietary fibers.

2. Materials and Methods

2.1. Materials

Experimental trials were carried out with brown wheat flour (cv. Bologna), which can be considered as type 2 according to Italian flour classification legislation (i.e. approx. 85% of extraction yield, maximum ash content = 0.95 g/100 g dm). The flour was processed with a stone grinding mill and a sieve (two consecutive passages through a 1,100–1,200 μm sieve) at the Molino Paciscopi (Montespertoli, Florence, Italy). Two batches of Bologna brown flour from the same year and geographical area (Montespertoli, Florence, Italy) were used in the two trials; their composition is reported in Table 1.

Table 1 T1 and T2 brown flour composition.

Trial	Flour	Starch (%)	Protein (g/100g)	Fiber (g/100g)	Ash (g/100g)	Moisture (%)
T1	batch 1	64.2	10.88	6.5	0.6	14.4
T2	batch 2	62.3	11.01	8.8	0.8	15.0

2.1.1. First trial (T1): bread doughs with different water content, with or without pre-gelatinized starch

This trial was carried out on dough recipes with three different levels of water (59%, 70% and 80% w/w total mass of flour) and two levels of GBF: (i) a control sample without GBF (T1-0%); and (ii) 6% GBF (T1-6%). GBF was expressed as the percentage weight of the flour that was used to prepare it with respect to the total mass of flour. Dough moisture content corresponded to (water weight)/(total dough mass)* 100. The mass balance of dough recipes is shown in Table 2.

The baking process was standardized, and is reported below. Rheological analyses of doughs were carried out using Farinograph (Brabender, Duisburg, Germany) and Alveograph (Chopin technologies, Villeneuve-la-Garenne, France). Bread quality was evaluated both immediately after baking, and 48 h after baking. Bread volume (L), bread specific volume (L/Kg), crumb and crust moisture (g/100 g), and instrumental bread texture were measured.

Table 2 Dough recipes for T1 and T2.

Sample	Water addition (%)	Total flour (g)	Total water (g)	Flour for GBF (g)	Water for GBF (g)	Total GBF (g)	Added flour (g)	Added water (g)	Yeast (g)	Dough (g)	Flour moisture content (%)	Yeast moisture content (%)	Dough moisture content (%)
T1-0%	58.9	310	183	-	-	-	310	182	13	505	14.4	66.5	46.6
T1-0%	70.0	310	217	-	-	-	310	217	13	540	14.4	66.5	50.0
T1-0%	80.0	310	248	-	-	-	310	248	13	571	14.4	66.5	52.8
T1-6%	58.9	310	183	18.6	74.4	93.0	291	108	13	505	14.4	66.5	46.6
T1-6%	70.0	310	217	18.6	74.4	93.0	291	143	13	540	14.4	66.5	50.0
T1-6%	80.0	310	248	18.6	74.4	93.0	291	174	13	571	14.4	66.5	52.8
T2-0%	61.0	310	189	-	-	-	310	189	13	512	15.0	66.5	47.7
T2-6%	64.5	310	200	18.6	74.4	93.0	291	126	13	523	15.0	66.5	48.8

2.1.2. Second trial (T2): bread doughs at a reference consistency with or without GBF

The same levels of GBF (i.e. 0 and 6%) used in T1 were tested again, but this time dough samples were prepared using the amount of water required to reach the reference farinograph consistency of 500 Brabender Units (BU). Baking, dough rheological analyses, and bread quality evaluation followed the method described in T1; in addition, crumb specific volume (mL/g) was determined.

2.2. Preparation methods

2.2.1. GBF processing

GBF was prepared following Kim et al. (2016), namely with a 1:4 ratio of brown flour to mineral water (Levissima, Bormio, Italy). The mixture was continuously stirred as it was heated to 85°C. This temperature was maintained for three min to complete starch gelatinization. Temperature was measured with a Type J penetration probe (Testo, Lenzkirch, Germany). GBF was cooled to room temperature and stored at 4°C; it was used in experimental trials the d after gelatinization.

2.2.2. Bread making

The straight dough method was applied. Mixing of ingredients, dough formation, resting, leavening with fresh brewer's yeast (Lievital, Trecasali, Italy), and baking were all carried out with a bread machine (Pain dorè, Moulinex, Ecully, France). Baking temperature profiles were measured using a Type J thermocouple (diameter 1 mm, RdF, Hudson, New Hampshire) connected to an automatic data acquisition and recording system (Datascan 7220, Newbury, UK) interfaced to a computer. After baking, bread samples were cooled to room temperature and stored in paper bags.

2.3. Measurement methods

2.3.1. Brown flour composition

Flour moisture (AACC 44-15.02), starch (M24.14.01), protein (ISTISAN 1996/34, N x 6.25), total dietary fiber (AOAC 985.29) and ash (ISTISAN 1996/34) were measured according to AACC International Approved Methods.

2.3.2. Rheological analysis

Dough rheology was measured using both a Brabender Farinograph and a Chopin Alveograph. In T1, the farinograph test was performed in duplicate to determine dough consistency (BU) for the three amounts of added water, with and without GBF. In T2, the same test was carried out in duplicate following the international standard method

(AACC No. 54-21) to determine the amount of water necessary to obtain dough samples with a reference consistency of 500BU with or without GBF.

The alveograph test was carried out in five replicates for both T1 and T2 following the AACC Method 54-30A (AACC, 2000), with some modifications. In order to predict dough performance during the baking process, doughs were prepared in the alveographic mixer following planned recipes (Table 2). Therefore, the amount of brown flour was constant, and corresponded to the value given in the standard method: 250 g for each sample. However, the amount of water added to the doughs did not correspond to the standard method, but was consistent with the % water content (w/w total mass of flour in the recipe) of T1 and T2 recipes (Table 2).

For all samples the following parameters were measured: (i) dough tenacity (P; mm H_2O); (ii) dough extensibility (L; mm); (iii) the ratio P/L; (iv) flour strength (“W”; 10^{-4} J); and (v) the swelling index (G; mm).

2.3.3. Bread quality measurement

Bread volume (L) was measured using the standard millet displacement method (AACC, 2000). Specific volume (L/kg) was determined as the ratio between total volume (L) and weight (kg). Crumb specific volume (mL/g) was determined by cutting a small piece of crumb (5–10 g) and determining the ratio between its volume (mL) (determined using the standard millet displacement method (AACC, 2000)) and its weight (g). Crumb and crust moisture (g/100 g) 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 (Stable Micro Systems, UK), equipped with a circular flat-plate probe (diameter: 30 mm), according to the procedure described in Kim, Kwak & Jeong (2017). Hardness (N), cohesiveness, gumminess (N), chewiness (N*mm) and springiness (mm) were measured for both trials. The TPA test was carried out on three slices (1.5 cm thickness) of each bread sample in five replicates.

2.3.4. Data processing

T1 followed a full factorial experimental design with three replicates. Tested factors were GBF at two experimental levels (0% and 6%), and water amount at three experimental levels (59%, 70% and 80%). A two-way ANOVA was performed in order to assess significant ($p<0.05$) differences due to these factors and their interaction. To assess differences due to bread staling, a three-way ANOVA was performed on parameters measured immediately, and 48 h after baking. In both cases, the Tukey HSD test was used as the post-hoc test.

T2 consisted of five replicates that compared the characteristics of doughs and breads at the reference consistency of 500BU. A t-test was performed in order to assess differences between mean values of the measured parameters. A two-way ANOVA was performed in order to assess significant ($p<0.05$) differences due to storage time, GBF, and their interaction. The Tukey HSD test was used as the post-hoc test.

3. Results and discussion

3.1.T1: bread dough with different water content, with or without GBF

Table 2 shows the mass balances of dough recipes. The 6% level of GBF was determined following preliminary tests in which 3%, 6%, 9% and 12% levels were applied (data not shown). Rheological test on doughs (specifically “W” and P/L alveographic parameters) and bread quality measurements (bread volume and bread hardness) revealed that 6% was the proper GBF amount to use in order to optimize the baking performance.

Recipes were designed in order to maintain the same dough amount and moisture content between samples with or without GBF, given flour and yeast moisture content. The three levels of water addition (58.9%, 70% and 80%) were chosen to produce a broad range of dough consistencies, then to study the effects of both water content and GBF on dough and bread samples. The above levels correspond to an effective dough moisture content (DMC, water weight/total dough mass*100) of 46.6%, 50% and 52.8%, respectively.

3.1.1. Dough rheology

Fig. 2 shows the dough consistency of samples as BU values from farinograph tests. Both water levels and GBF greatly affected consistency, which decreased as the water content increased. Moreover, BU values for all samples containing 6% GBF were significantly higher than samples without GBF ($p=0.036$). In particular, T1-0% samples ranged from 560BU for the least hydrated sample, to 100BU for the most. A similar trend was observed for T1-6% samples, but consistency values were higher (ranging from 620BU to 150BU as DMC increased from 46.6% to 52.8%).

Alveograph parameters are important to predict baking performance, as the alveograph test causes deformations that are similar to those that occur during dough leavening and baking (Zhou, Therdthai, & Hui, 2012). Fig. 3 shows “W” and P/L values; these parameters are the best predictors of breadmaking performance. High “W” values are associated with a good bake, and the ratio between dough tenacity and elasticity (P/L) has to be well-balanced. For refined flours, the optimal reference is 0.4–0.7 (Quaglia, 1984). In unrefined flour P/L values are usually higher (Cappelli et al., 2018; Parenti et al., 2013). Hence, to improve the baking performance of unrefined flours, it is necessary to minimize the P/L parameter.

Both the water level ($p<0.001$) and starch gel ($p<0.001$) affected significantly flour strength (“W”), which decreased as DMC increased (Fig. 3). DMC was highest in samples with the lowest strength. Moreover, “W” was highest in dough containing GBF. In particular, “W” values for T1-0% samples were approximately 81 10^{-4}J for the lowest DMC, and 44 10^{-4}J at 50% DMC. In comparison, “W” values for T1–6% samples were approximately 88 10^{-4}J for the lowest DMC, and 67 10^{-4}J at 50% DMC. At 52.8% (the highest DMC studied) the dough of T1-0% samples did not properly develop, and it was neither workable nor measurable. On the other hand, it was possible to obtain and measure T1-6% samples. In this case, “W” was approximately 23 10^{-4}J. This demonstrates that adding GBF significantly increased dough strength ($p<0.001$).

The GBF –DMC interaction showed a significant ($p=0.009$) effect of the experimental P/L values. An increase in DMC significantly reduced P/L values in both T1-0% and T1-6% samples. GBF also significantly influenced P/L values, which were higher than T1-0% samples for each DMC investigated. P/L values for T1-0% samples were approximately 2.2 at 46.6% DMC, and approximately 0.4 at 50% DMC; GBF samples started from approximately 2.8 for the least hydrated sample, decreasing to approximately 1.4 P/L at 50%. As noted above, at the highest studied DMC (52.8%) the dough of the T1-0% samples could not be prepared, while a P/L value of approximately 1 was measured for T1-6% samples. Hence, to optimize (i.e. minimize) the value of P/L, different water contents were required for T1-0% and T1-6%.

These rheological results confirm the well-known importance of water content for good dough development, notably gluten network formation (Zhou, Therdthai, & Hui, 2012), and they demonstrate that GBF had a marked effect on dough consistency. It is also important to point out that dough rheology was strongly affected by a narrow range (46.6–52.8%) of water content (Table 2).

The mechanisms that govern how water content influences dough rheology are related to the molecular mobility of water (Blanshard et al., 1985). The addition of GBF may influence water distribution in the dough. At room temperature, starch granules are able to absorb water up to about 50% of their dry weight, but the gelatinization process increases this through granule swelling and the loss of crystallinity and molecular order

(Goesaert et al., 2005). Consequently, in GBF enriched samples, water binding to starch molecules could mean that there is less available for dough development; at the same moisture level, GBF doughs had higher consistency than T1-0% samples. Hence, in the presence of pre-gelatinized starch, the same dough consistency could be obtained with more water.

Alveograph data clarified the influence of water content and GBF on the dough's physical and mechanical properties. A certain amount of water is essential for protein hydration, which optimizes gluten network development and creates a perfect balance between tenacity, elasticity and extensibility (Zhou, Therdthai, & Hui, 2012). The experimental "W" and P/L values that were observed as a function of water content could be consistent with the above phenomena. The addition of GBF significantly increased P/L values, and this could be due to an increase in tenacity (i.e. P) and/or a decrease in extensibility (i.e. L). The gelatinization process could be responsible for making water less available for gluten hydration (Blanshard et al., 1985).

3.1.2. Bread quality

Table 3 shows quality characteristics of bread samples. Water content exercised a significant effect on crumb moisture, cohesiveness and springiness. Crumb moisture clearly increased as water content increased, and higher DMC was consistent with higher crumb moisture. Cohesiveness refers to how well the product withstands a second deformation relative to its resistance under the first deformation. This also increased as a function of water content; an increase of approximately 70% was seen as DMC increased from 46.6% to 52.8%. Furthermore, springiness increased. Springiness is an indicator of the product's elasticity (i.e. how well it physically springs back after it has been deformed during the first compression). This parameter increased by approximately 20% between samples with 46.6% and 52.8% DMC.

The addition of GBF had a significant effect on crust moisture (Table 3). Experimental data showed a decrease of approximately 10% for T1-6% samples compared to T1-0%. GBF could facilitate water mass transfer at the bread's surface; absorbed water in the starch could be more "free" than water in the development of the gluten network, leading to greater crust dehydration in the T1-6% samples.

Significant effects of the GBF –water interaction can be observed with respect to bread volume, and hardness parameters (Table 3). Regarding bread volume, at 46.6% DMC, bread volumes of T1–0% samples were significantly higher than T1–6% samples. At 50% DMC, a significant increase in bread volume was observed in all samples, compared to 46.6%. However, at 52.8% DMC, the volume of T1-0% samples fell (to 1.32 L), while T1–6% samples reached their highest value (1.4 L). Therefore, maximum bread volume occurred at 50% DMC in T1-0% samples and at 52.8% DMC in T1-6% samples. With respect to crumb hardness, in T1-0% samples this parameter was lower at 50% DMC than at 46.6%. On the other hand, it increased at 52.8% DMC to a value higher than at 46.6% (5.1 compared to 3.4), indicating a non-linear trend. T1-6% samples differed, as hardness decreased linearly as water content increased. In this case, samples were hardest (8N) at 46.6% DMC; this decreased to 5.8N at 50% DMC, reaching 3N at 52.8% DMC. T1-0% and T1-6% different trends, with regard to bread volume and hardness, revealed that they had different water requirements. Specifically, a DMC around 50% could optimize T1-0% bread quality (i.e. highest volume, lowest hardness), while higher water content was necessary to optimize T1-6% sample. Hence, GBF addiction significantly changed (i.e. enhanced) the optimum water amount of bread doughs.

These results are consistent with the literature (Kim, Kwak & Jeong, 2017; Zettel et al., 2014; Yamauchi et al., 2014). Kim, Kwak & Jeong, (2017) reported a decrease in hardness in rice bread samples prepared with GBF. Yamauchi et al. (2014) observed that bread samples containing GBF were softer than control ones.

The above quality characteristics were compared with those for samples stored for 48 h at room temperature (simulating shelf-life). The only significant difference was found for crumb hardness with respect to T1–0% samples. In this case, at 46.6% DMC crumb hardness increased from 3.4±0.3 to 5±1; at 50% it increased from 2.4±0.1 to 4.9±0.2, and at 52.8% DMC it increased from 5.1±0.4 to 6.4±0.8. No significant differences were found for T1-6% samples. Therefore, the addition of GBF also reduced staling, and helped to preserve bread softness during shelf-life.

Overall, the results of T1 demonstrated a significant effect of GBF on both dough rheology and bread volume and texture. This effect was directly related to DMC, suggesting that bread quality can be optimized by adjusting this parameter. The following section addresses this question.

Table 3 Quality characteristics of T1 bread

Parameter	T1-0% samples			T1-6% samples			P GBF	P H_2O	P GBF*H_2O
	46.6% DMC	50% DMC	52.8% DMC	46.6% DMC	50% DMC	52.8% DMC			
Bread volume (L)	1.33 ± 0.07	1.4 ± 0.2	1.32 ± 0.04	1.21 ± 0.05	1.30 ± 0.09	1.4 ± 0.2	ns	ns	*
Bread specific volume (L/kg)	3.31 ± 0.16	3.27 ± 0.24	2.95 ± 0.09	3.00 ± 0.11	3.01 ± 0.20	3.13 ± 0.36	ns	ns	ns
Crumb moisture (g/100 g)	44.8 ± 0.6^{x}	49.3 ± 0.2^{y}	52.2 ± 0.1^{z}	45.1 ± 0.5^{x}	49.6 ± 0.2^{y}	52.2 ± 0.2^{z}	ns	***	ns
Crust moisture (g/100 g)	27 ± 3^{a}	26.1 ± 0.8^{a}	28.9 ± 0.4^{a}	23.2 ± 0.6^{b}	25.4 ± 0.3^{b}	26.3 ± 0.3^{b}	*	ns	ns
Hardness (N)	3.4 ± 0.3^{a}	2.4 ± 0.1^{a}	5.1 ± 0.4^{a}	8 ± 1^{b}	5.8 ± 0.6^{b}	3 ± 1^{b}	**	ns	**
Hardness (N) 48h	5.2 ± 1.1	4.9 ± 0.2	6.4 ± 0.8	14.4 ± 7.4	4.6 ± 0.3	4.4 ± 0.3	ns	ns	***
Cohesiveness	0.24 ± 0.03^{x}	0.34 ± 0.01^{x,y}	0.41 ± 0.06^{y}	0.27 ± 0.02^{x}	0.33 ± 0.05^{x,y}	0.46 ± 0.08^{y}	ns	*	ns
Springiness (mm)	0.72 ± 0.06^{x}	0.83 ± 0.10^{x,y}	0.92 ± 0.02^{y}	0.75 ± 0.03^{x}	0.83 ± 0.06^{x,y}	0.91 ± 0.02^{y}	ns	**	ns
Chewiness (N mm)	0.58 ± 0.07	0.81 ± 0.17	1.91 ± 0.26	1.50 ± 0.24	1.60 ± 0.33	1.36 ± 0.37	ns	ns	ns

Data are expressed as mean± SE. DMC is dough moisture content (water weight/total dough mass*100). P GBF, P H_2O and P GBF * H_2O refer to the effects of these factors: GBF, water and their interactions GBF*water. *, ** and *** indicate significant differences at $p<0.05$, $p<0.01$ and $p<0.001$, respectively; "ns" indicates no significant difference at $p<0.05$. Means in a row with different superscripts are significantly different ($p<0.05$); specifically, "a", and "b" refer to the GBF main effect, while "x", "y" and "z" refer to the water main effect.

3.2.T2: bread dough at a reference consistency, with or without GBF

The literature suggests that optimal dough consistency is 500BU for refined flours. Other Fig.s have been reported for unrefined flours, due to their different composition (Zhou, Therdthai, & Hui, 2012). A higher fiber content increases water absorption, and competition for hydration with protein molecules during dough development (Gómez et al., 2003). Nevertheless, even in this case, 500BU remains the value that is typically used to determine optimal dough development (Boita et al., 2015; Khalid, Ohm & Simsek, 2017). Therefore, we compared dough rheology and bread quality between samples without GBF (T2-0%) and with GBF (T2-6%) at a dough consistency of 500BU.

3.2.1. Dough rheology

A Farinograph test was carried out, in order to determine the amount of water necessary to reach the reference consistency of 500BU. This found that the T2-0% samples required 61% water (w/w total flour mass in the recipe), while the T2-6% samples required 64.5%. These levels correspond to 47.7% DMC for T2-0% dough samples and 48.8% DMC for T2-6% samples (Table 2). These experimental data were congruent with those obtained during the T1 trial; T2-6% dough samples required more water to reach the same consistency as T1-6% sample, as GBF reduced the availability of water for dough development. No significant differences between T2-0% and T2-6% samples were found with regard to the other farinographic parameters: DDT – dough development time, 5±0.5 min; DST – dough stability, 10±1 min; DW – dough weakening 20±5BU.

Alveograph experimental data highlighted significant differences between T2 samples; Fig. 4 shows flour strength (“W”), and P/L. Dough extensibility increased by approximately 30%: from 35±3mm for T2-0% samples to 46±4mm for T2-6% samples. A similar increase of approximately 30% was found for “W”: from 73±7 10^{-4}J for T2-0% samples to 96 ± 5 10^{-4}J for T1-6% samples. On the other hand, P/L did not change significantly (1.4±0.2 compared to 1.1±0.2; $p=0.09$). In T2 the additional water content improved the alveograph performance of dough compared to T1. Moreover, in T2, “W” reached the same, maximum value observed in T1, while P/L remained low. These results suggest that the use of GBF improves baking performance, as the capacity of the dough to hold gas during the leavening step is improved, which, in turn, probably increases the volume, and softens the texture of bread.

3.2.2. Bread quality

Fig. 1 shows the baking temperature profiles of T2-0% and T2-6% samples, which were consistent with bread baking theory (Zanoni, Peri & Pierucci, 1993). Despite the different water amount of T2-0% and T2-6% samples (61% vs 64.5%, w/w total flour mass in the recipe), no significant differences were found in the baking ramp profile.

Table 4 shows quality characteristics of bread samples immediately after baking and after 48 h of storage at room temperature.

Immediately after baking, crumb moisture content for all T2 samples was congruent with DMC values given in Table 2. Bread volume was approximately 20% higher in T2-6% samples than in T2-0% samples. Another clear effect was found for crumb specific volume, which increased by approximately 15% for T2-6% samples compared to T2-0%. Fig. 5 shows that GBF led to more swollen and porous bread samples. These experimental data are consistent with Yamauchi et al. (2014), Zettel et al. (2014) and Kim, Kwak & Jeong (2017), who observed that the addition of GBF caused a significant increase in bread volume. Therdthai, Zhou & Adamczak (2002) and Tsai et al. (2012) argue that highly gelatinized dough increases bread volume by retaining more gas and water vapor in pore walls. Naito et al. (2005) suggest that increased amounts of GBF provide wall materials with stickiness and good expansion characteristics.

All texture parameters changed significantly with the addition of GBF. T2-6% samples were characterized by the following; lowest hardness, highest cohesiveness, and lowest springiness. In particular, hardness decreased by approximately 35% (Kim, Kwak & Jeong 2017; Zettel et al., 2015; Yamauchi et al., 2014), while springiness decreased by approximately 50%.

Storage was associated with staling (Table 4). After 48 h, significant decreases in crumb specific volume, crust moisture and cohesiveness were found for all samples. Consistent with the literature (Zettel et al., 2015; Yamauchi et al., 2014), differences between T2-6% and T2-0% samples were preserved during storage with respect to crumb specific volume and texture parameters. On the other hand, hardness increased more in T2-0% samples than T2-6% samples. Finally, differences in springiness were observed between the samples; it decreased in T2-0% samples, while it increased in T2-6% samples, reaching values close to those of bread without GBF immediately after baking.

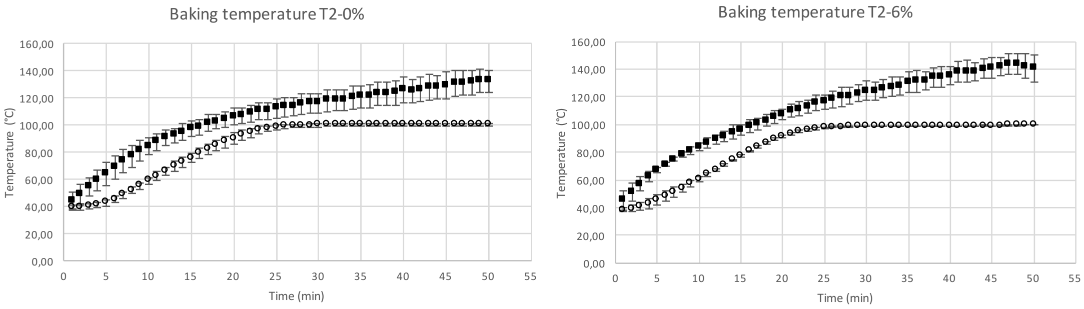

Fig. 1 Bread quality. The baking temperature profiles (Fig. 1) of the T2-0% and T2-6% samples were similar. Black squares represent crust temperature; white circles represent crumb temperature. According to the bread baking theory (Zanoni et al., 1993), a higher temperature than 100°C, which asymptotically tends towards the oven temperature, was reached at the bread's surface; then, a dehydration occurred and a dried and brown crust was formed (Table 3). At the inner bread's portion, the temperature rose at low rate and asymptotically tended towards 100°C (i.e. the evaporation-front temperature); then, a crumb was formed with a moisture content which was the same value as that of the raw dough (Table 3).

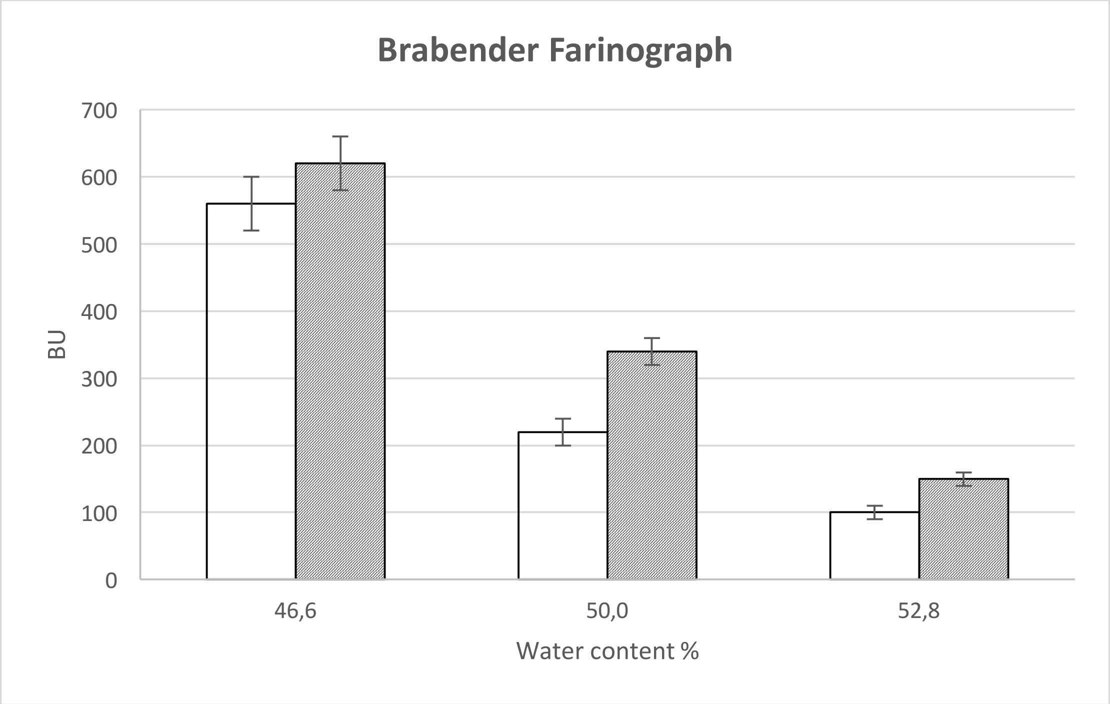

Fig. 2 Farinographic test on T1-0% and T1-6% samples at different dough moisture contents: 46.6%, □ 50.0%, ▨ 52.8%; – T1-0%; – T1-6%.

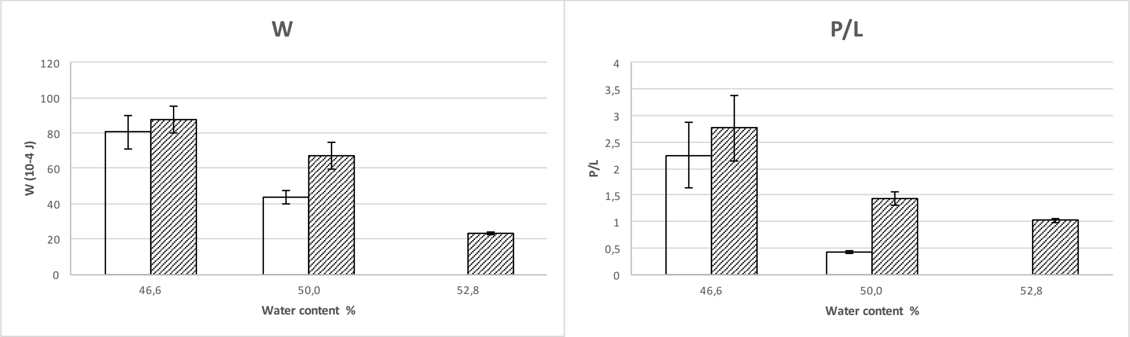

Fig. 3 Alveographic parameters W (10^{-4}J), the baking strength and P/L, the ratio between tenacity (P) and extensibility (L) of T1-0% and T1-6% samples at different dough moisture contents: 46.6%, 50.0%, 52.8%; white bars represent T1-0% samples; striped bars represent T1-6% samples.

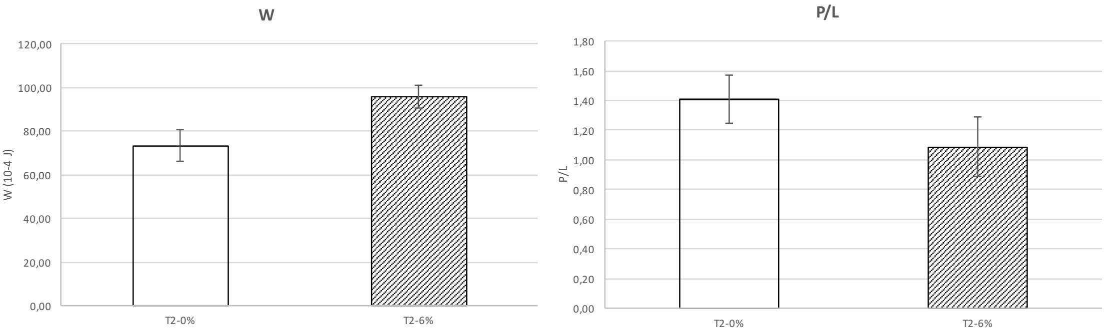

Fig. 4 Alveographic parameters: “W” (10^{-4} J), the baking strength, L (mm), extensibility and P/L, the ratio between tenacity (P mm H_2O) and extensibility L (mm) of T2-0% and T2-6% samples at the optimal consistency value of 500BU; white bars represent T2-0% samples; striped bars represent T2-6% samples.

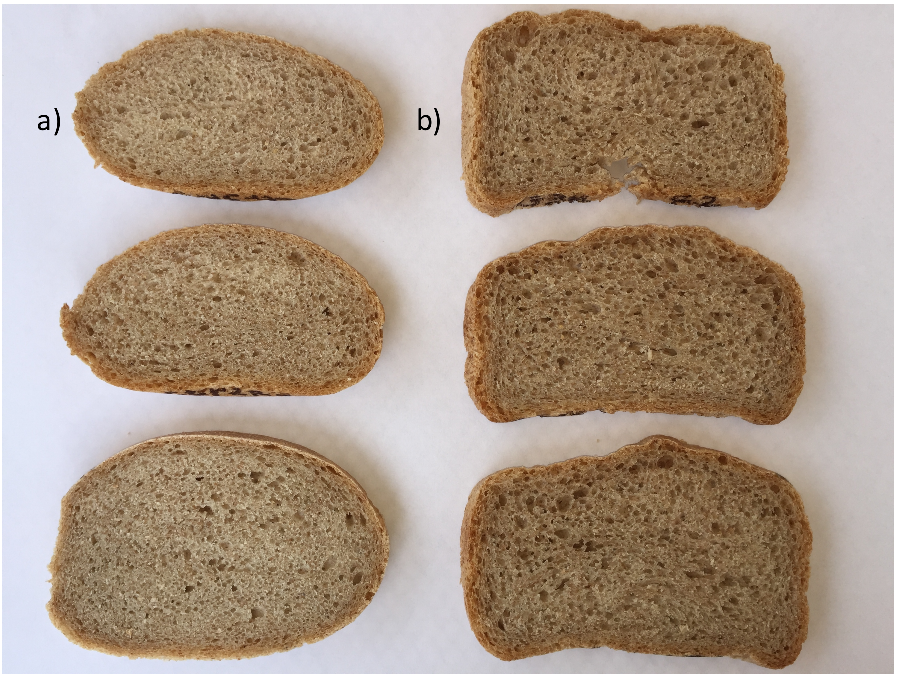

Fig. 5 Bread structure of T2-0% (a) on the left vs T2-6% (b) on the right.

4. Conclusions

To the best of our knowledge, this is the first study of the effect of GBF to bread and dough made with brown flour. It is found to have several effects. First, it interacts strongly with water, changing the amount of water required to make dough. The correct dose of GBF is linked to the optimal amount of water; in this trial, we chose a reference value of 500BU. GBF significantly improved both the dough's rheological proprieties and bread quality. Better strength ("W") and tenacity/extensibility ratio (P/L) were obtained with the addition of 6% GBF. Furthermore, both bread volume and texture were significantly improved. The 48 h storage test confirmed that changes due to GBF can be maintained during the bread's shelf-life. GBF technique is based on the incorporation of standard brown flour; only its form changes. As it does not involve the use of additives, the high nutritional value of the flour is preserved. Furthermore, as it produces brown breads with better quality parameters, it could be used to promote the adoption of unrefined, healthier foods.

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4.3 Two-step approach for bread formula optimization

4.3.1 Preliminary remark – Article 4

The research study of the Doctoral thesis continued focusing on the bread dough formula. We have already reported that the scientific research aimed to improve the bread-making performance of unrefined wheat flours has mainly focused on the modification of the bread formula. In detail, the effects of improvers such as enzymes, emulsifiers, hydrocolloids, oxidants and other functional ingredients have been explored for unrefined bread formulas (Tebben et al., 2018; Farbo et al., 2020; Parenti et al., 2020a). Conversely, the effects of the most common improvers usually added in the bread-making formula has not been investigated since their effects are widely described in the scientific literature and are considered as references. However, the reported effects of these common bread improvers have been obtained on the bread-making performance of refined flours which are characterised by different chemical composition compared to unrefined wheat flours. Hence, it is reasonable to expect that the effects of the improvers on the technological properties of unrefined wheat flours may be different than those observed on refined wheat flours. As a consequence, the results obtained on refined wheat flours can not be extended to unrefined wheat flours since the raw materials used in the bread formula are different. Furthermore, the chemical composition of refined flours is less complex and less susceptible to deterioration phenomena compared to unrefined flours, both features that make the results obtained on some refined flour samples more appropriately appliable to all the refined flour category, despite the inherent variability due to wheat genotype and environmental conditions. Conversely, since the chemical composition of unrefined flour category can markedly change as a function of several factors (i.e. the amount and typologies of milling by-products, milling method, wheat genotype, environmental conditions, flour evolution during storage etc.), reference effects of the improvers for the unrefined wheat flour category can not be identified. Therefore, we thought that a different approach could probably be more appropriate for the evaluation of the thecnological properties of unrefined wheat flour category. Furthermore, we also realized that in the literature the effect of improvers was assessed using one of them at a time, while in industry combinations of improvers are usually adopted. Thus, we designed the experiment to test the simultaneous effect of different improvers on the bread-making performance of an unrefined wheat flour.

These arguments drove the thesis research to the investigation of a new approach, based on the DOE, that should be able to optimize the bread formula of an unrefined wheat flour sample. We divided the study in two steps; in the first step the simultaneous effects of different common and natural bread ingredients, including gelatinized wheat flour (tested in article 3, Parenti et al., 2019), on the bread-making performance of an unrefined flour variety were tested. The improvers that showed the best results on the bread quality were then selected for the following step, where an in-depth evaluation of the improvers was performed in order to optimize the bread recipe according to the criterion of minimizing the addition of the ingredients. The aim of this study was to propose a different approach for improving the bread-making performance of unrefined wheat flours, based on a two-step optimization of the bread formula.

4.3.2 Article 4

Parenti, O., Guerrini, L., Cavallini, B., Baldi, F., & Zanoni, B. (2020). Breadmaking with an old wholewheat flour: Optimization of ingredients to improve bread quality.
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Breadmaking with an old wholewheat flour: optimization of ingredients to improve bread quality

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Abstract

Processing strategies are necessary to improve the quality of baked old wholewheat flour products, since they are required by consumers but have poor technological properties. The present study tested the addition of common improvers on an old wholewheat flour performance to optimize bread quality. At first, the effect of seven improvers on dough rheology and bread specific volume was evaluated using a screening design method. All of the improvers affected the farinographic parameters; the most promising effects were shown by sucrose, salt and guar gum. Bread specific volume was significantly improved by sucrose, extra virgin olive oil and ice; hence, the effects of these variables on dough rheology and bread quality were evaluated in-depth in a full factorial trial. Dough stability and dough weakening were significantly improved by sucrose and extra virgin olive oil. Sucrose and extra virgin olive oil interaction optimized bread specific volume, crumb specific volume and hardness. The addition of 2% sucrose and 3% extra virgin olive oil resulted in optimized bread, on which a qualitative sensory evaluation was performed. This optimization approach could be applied to other wholewheat flours to improve product quality, hence promoting the consumption of high nutritional value breads.

Key words
unrefined flour, bread improvers, Brabender Farinograph, healthy foods

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1. Introduction

Wheat bread represents the staple food in many diets, with a far-reaching impact on human health. Depending on the degree of refinement of the flour used in the bread recipe, the composition of the final product changes immensely.

Refined flours are mainly composed of the starchy endosperm, while they are deprived of the germ fraction and the outer kernel layers. Conversely, unrefined flours are extremely rich in compounds such as dietary fibres, fats, minerals, vitamins, lignans and phenolic compounds, which are positive for human health (Zhou et al., 2014).

In recent years, several scientific studies have shown that a regular consumption of wholewheat products protects from chronic diseases such as cardiovascular disease, type 2 diabetes and some types of cancers (Ye, Chacko, Chou, Kugizaki, & Liu, 2012). Unfortunately, unrefined flours show a poor technological performance, since the presence of the bran fraction has a negative effect on the breadmaking process, and changes the taste and flavour of the resulting bread (Gómez, Ronda, Blanco, Caballero, & Apesteguía, 2003). Therefore, refined wheat flour still represents the preferred choice for bread production.

Due to increasing consumer attention towards healthy food, in the recent years there has been renewed interest in old wheats (Guerrini et al., 2019). Old wheats are generally defined as those wheat varieties cultivated before the intense genetic selection that took place during the Green Revolution of the 1960s (Dinu, Whittaker, Pagliai, Benedettelli, & Sofi, 2018). Hence, the old wheat term includes a large number of cultivars, with a broad genetic base, and therefore showing a broad range of characteristics (Dinu, Whittaker, Pagliai, Benedettelli, & Sofi, 2018; Mefleh, Conte, Fadda, & Giunta, 2018). Within them, some varieties were reported to have high nutritional value and potential health benefits (Leoncini et al., 2012; Dinelli et al., 2011; Sofi et al., 2010; Gotti et al., 2018; Sereni et al., 2016).

Considering the poor technological properties of old wholewheat flours compared to conventional flour blends, it is still a challenge to use them in breadmaking (Cappelli et al., 2018; Fabro et al., 2020). Thus, different operating procedures should be specifically designed to maximize the technological performance of old wheat flour, for example by using some ingredients in the recipe with the aim of ameliorating the final product quality (i.e. improvers).

In this study an optimization approach was carried out to find the best combination of improvers employed in breadmaking on sp. Triticum aestivum L., cv. Verna old wholewheat flour, evaluating their effects on the quality of dough and bread. At first, seven common improvers were evaluated following an optimized experimental design, in order to reveal which of them had the greatest effect on bread quality. This evaluation enabled the selection of three bread improvers, which were evaluated in-depth in a full factorial design trial. Finally, an optimized bread recipe was identified.

2. Materials and methods

2.1 Materials

Experimental trials were carried out with two batches (V1 and V2 batches) of a sp. Triticum aestivum L., cv. Verna; wheat seeds were grown in Montespertoli (Florence, Italy), during the growing season 2018-2019. The chemical and physical characterization of the old wholewheat flour Verna batch 1 "V1" and Verna batch 2 "V2" was as follows: moisture (V1=12.83g/100g~~%~~, V2=13.46g/100g~~%~~), ash (V1=1.01g/100g d.m., V2=1.28g/100g d.m.) and protein (V1=12.3g/100g d.m., V2=10.5g/100g d.m.) contents; WA (V1=57.75%, V2=55.00%), DDT (V1=3.00min, V2=2.50min), DS (V1=2.00min, V2=1.17min) and DW (V1=165BU, V2=203BU); P (V1=39.0mmH_2O, V2=46.0mmH_2O), L (V1=30.0mm, V2=25.0mm) and W (V1=42.3 10^{-4}J, V2=44.8 10^{-4}J) and P/L (V1=1.3, V2=1.9).

The old wholewheat flours were processed using a stone grinding mill and a sieve (two consecutive passages through a 1,100–1,200 μm sieve) at the Molino Paciscopi (Montespertoli, Florence, Italy). Mineral water (Levissima, Bormio, Italy), fresh brewer's yeast (Lievital, Trecasali, Italy), extra virgin olive oil (EVOO), guar gum (GG), sucrose (Suc) and sodium chloride (NaCl) were purchased at a local market (Florence, Italy). Ascorbic acid (AH_2) was purchased in a drugstore. Ice (prepared with the above mineral water) and gelatinized flour (GF) were prepared in the lab the day before each trial. The GF was prepared with a 1:4 ratio of old wholewheat flour to mineral water (Levissima, Bormio, Italy). The mixture was continuously stirred as it was heated to 85 °C for 3 min. Temperature was measured with a Type J penetration probe (Testo, Lenzkirch, Germany). GF was cooled to room temperature, stored at 4 °C and used the following day as bread improver (Parenti et al. 2019).

2.2 The experimental design

2.2.1 The screening design trial (T1)

A Plackett-Burman screening design (Antony, 2014) was adopted to simultaneously test the main effects of the seven bread improvers on dough performance and bread quality. The screening design allowed the seven factors to be tested at two levels using only eight samples. The chosen variables, their level settings and the combinations used in the eight trials are shown in Table 1.

The T1 trial was carried out on the V1 Verna old wholewheat flour batch. Rheological analyses of doughs were carried out using a Farinograph (Brabender, Duisburg, Germany). The baking process was standardized as reported below. Bread quality was evaluated by measuring the bread specific volume immediately after baking.

Table 1. T1 trial settings; gelatinized flour = GF; extra virgin olive oil = EVOO, sucrose = Suc, ascorbic acid = AH_2, guar gum = GG. The symbol "-" represents the lowest level of each factor (i.e. 0%), the symbol "+" represents the highest level of each factor, which is shown in the table.

Samples	GF (6%, w/ flour w)	EVOO (3%, w/ flour w)	Suc (6%, w/ flour w)	AH_2 (100 ppm)	GG (1%, w/ flour w)	NaCl (2%, w/ flour w)	Ice (20%, w/ water w)
1	+	+	+	−	−	−	+
2	−	+	+	+	+	−	−
3	+	−	+	+	−	+	−
4	−	−	+	−	+	+	+
5	+	+	−	−	+	+	−
6	−	+	−	+	−	+	+
7	+	−	−	+	+	−	+
8	−	−	−	−	−	−	−

Table 2. Farinographic parameters of T1 trial dough samples with addition "+" or not "-" of the seven improvers.

FACTOR	WA (%)	P WA	DDT (MIN)	P DDT	DS (MIN)	P DS	DW (BU)	P DW
GF +	56.81 ± 1.03^{a}	*	2.50 ± 0.01^{a}	***	4.37 ± 0.41^{a}	n.s.	118 ± 12^{a}	n.s.
GF -	55.19 ± 1.03^{b}		3.37 ± 0.01^{b}		4.44 ± 0.41^{a}		101 ± 12^{a}	
EVOO +	55.25 ± 1.03^{a}	*	2.87 ± 0.01^{a}	***	4.31 ± 0.41^{a}	n.s.	102 ± 12^{a}	n.s.
EVOO -	56.75 ± 1.03^{b}		3.00 ± 0.01^{b}		4.50 ± 0.41^{a}		117 ± 12^{a}	
Suc +	54.94 ± 1.03^{a}	**	2.25 ± 0.01^{a}	***	4.87 ± 0.41^{a}	**	94 ± 12^{a}	**
Suc -	57.06 ± 1.03^{b}		3.62 ± 0.01^{b}		3.94 ± 0.41^{b}		126 ± 12^{b}	
AH_2 +	55.56 ± 1.03^{a}	n.s.	2.87 ± 0.01^{a}	***	3.94 ± 0.41^{a}	**	116 ± 12^{a}	n.s.
AH_2 -	56.44 ± 1.03^{a}		3.00 ± 0.01^{b}		4.87 ± 0.41^{b}		103 ± 12^{a}	
GG +	56.86 ± 1.03^{a}	*	3.50 ± 0.01^{a}	***	4.87 ± 0.41^{a}	**	107 ± 12^{a}	n.s.
GG -	55.37 ± 1.03^{b}		2.37 ± 0.01^{b}		3.94 ± 0.41^{b}		112 ± 12^{a}	
NaCl +	55.19 ± 1.03^{a}	*	3.37 ± 0.01^{a}	***	6.69 ± 0.41^{a}	***	63 ± 12^{a}	***
NaCl -	56.81 ± 1.03^{b}		2.50 ± 0.01^{b}		2.12 ± 0.41^{b}		156 ± 12^{b}	

Selected factors: gelatinized flour = GF; extra virgin olive oil = EVOO, sucrose = Suc, ascorbic acid = AH_2, guar gum = GG and salt = NaCl. Experimental data are expressed as mean ± standard deviations. *, ** and *** indicate significant differences at $p<0.05$, $p<0.01$ and $p<0.001$, respectively. "n.s." indicates no significant difference at $p<0.05$. Means in column with different superscripts are significantly different at $p<0.05$. Specifically, "a" and "b" refer to main effect of each factor.

Table 3. T2 trial settings showing all 18 variable combinations. The variables tested in T2 were: sucrose = Suc (3 levels: 0%, 2% and 4%, w/flour w); extra virgin olive oil = EVOO (2 levels: 0% and 3%, w/flour w) and Ice (3 levels: 0%, 10% and 20%, w/water w).

Sample	Suc (w/flour w)	EVOO (w/flour w)	Ice (w/water w)
1	0%	0%	0%
2	0%	3%	0%
3	0%	0%	10%
4	0%	3%	10%
5	0%	0%	20%
6	0%	3%	20%
7	2%	0%	0%
8	2%	3%	0%
9	2%	0%	10%
10	2%	3%	10%
11	2%	0%	20%
12	2%	3%	20%
13	4%	0%	0%
14	4%	3%	0%
15	4%	0%	10%
16	4%	3%	10%
17	4%	0%	20%
18	4%	3%	20%

2.2.2 The full factorial design trial (T2)

The screening design made it possible to evaluate a large number of factors with a small number of tests. However, there are several limitations. Specifically, the design is a resolution III design (Antony, 2014), meaning that the main effects could be confused with two-factor and higher order interactions. Hence, the three variables with the highest impact on bread quality in T1 were tested in detail in a validation trial (T2), following a full factorial design. The experimental design is shown in Table 3. The chosen maximum level of EVOO (2%) and Ice (20%) was the same as in the T1 trial, while the chosen maximum level of Suc was lowered from 6% to 4%. This choice was made since the addition of 6% Suc resulted in the excessive browning of the bread crust and the perception of too much sweetness during the bread tasting, while 4% Suc did not show these drawbacks (data not shown). Moreover, a medium level of Suc (i.e., 2%) and Ice (i.e., 10%) was also included.

The T2 trial was carried out on the V2 Verna old wholewheat flour batch. Rheological analyses of the dough were carried out using a Farinograph (Brabender, Duisburg, Germany). The baking process was standardized as reported below. The bread quality parameters were evaluated immediately after baking. Bread specific volume, crumb specific volume, crumb and crust moisture, instrumental bread texture (Texture Profile Analysis - TPA), crumb image analysis and bread colour were evaluated. A sensory evaluation was also carried out on the optimized sample.

2.3 Preparation methods

2.3.1 Breadmaking

The bread dough was prepared in 500g batches. The basic formulation was: flour (310g), fresh brewer's yeast (13g) and the amount of water required to reach the farinograph consistency value of 500BU (51-59.5%, w/flour w). The straight dough method was applied.

The improvers were added together with the main ingredients. The GF was warmed to room temperature, the Ice was finely broken up in a mixer and the AH$_2$ was carefully solubilized in mineral water before adding the improvers to the bread dough. The breadmaking phases were all carried out with a bread machine (Pain doré, Moulinex, Ecully, France) using the WWF programme (mixing step: 25min at room T, resting and leavening: 1h and 20min at 40°C, baking: 55min at 180°C). The bread samples were cooled to room temperature prior to the bread quality evaluation. Two replicates were performed in the T1 trial, and four in the T2 trial.

2.4 Measurement method

2.4.1 Chemical characterization of old wholewheat flour

Moisture (AACC 44–15.02), protein (ISTISAN 1996/34, N x 6.25) and ash (ISTISAN 1996/34) contents were measured according to AACC International Approved Methods.

2.4.2 Large deformation tests

Old wholewheat flour rheological characterization was performed according to the official method using a Farinograph (AACC 54-21.02) and Alveograph (AACC 54-30.02). Dough farinographic analyses were carried out in two replicates in the T1 trial and three replicates in the T2 trial.

2.4.3 Bread quality measurements

Bread volume (L) was measured using the standard millet displacement method (AACC, 2000). Specific volume (L/kg) was determined as the ratio between total volume and mass. Crumb specific volume (L/kg) was determined by cutting a small piece of crumb (5–10 g) and determining the ratio between its volume (L) (calculated using the standard millet displacement method (AACC, 2000)) and its mass (kg).

Crumb and crust moisture (g/100 g) were measured by gravimetry at 105 °C until constant weights were reached. Since the dough was prepared with different amounts of water (i.e. the quantity to reach 500BU), comparison between moisture parameters was made using the ratio between the crumb or crust bread moisture (g/100g) and the original dough moisture (g/100g).

The Texture Profile Analysis (TPA) of the bread samples was carried out by two-bite compression using a Texture Analyzer (Stable Micro Systems, UK), equipped with a circular flat-plate probe (diameter: 30 mm). Hardness (N), cohesiveness, gumminess (N), chewiness (N*mm) and springiness (mm) were measured on three slices (1.5 cm thickness) of each bread sample in five replicates.

Crumb porosity was evaluated by digital image analysis (Image J software, Color Inspector 3D.jar). Images of the central bread slice (thickness 1.0 cm) were acquired at a resolution of 1.2MP. Rectangular sections of the bread crumb were selected, converted into an 8bit grey scale and subjected to spatial calibration before the analysis. The threshold was chosen according to Gonzales-Barron & Butler (2006), using the Otsu method. The following measurements were determined: pore area at the 50th percentile (mm^2), and total pore area (%), determined as the ratio between the total pore area (mm^2) in the analysed bread crumb section and the total area of the analysed bread crumb section (mm^2). Three replicates were performed on each bread sample. Crumb and crust colour were determined by digital image analysis. Photos of the bread samples were taken in standard light conditions. The crumb colour was evaluated on the central slice of the bread, while crust colour was assessed on the upper surface of the bread. L* or lightness (black 0/white 100), a* (green-/red+) and b* (blue-/yellow+) values were calculated according to (CIE Commission, 1978). All measurements were carried out in triplicate.

2.4.4 Bread sensory evaluation - a descriptive analysis

The sensory profile of the optimized sample was compared to the control sample (i.e. bread without improvers - CTR) and a qualitative analysis was performed (Dinnella, Borgogno, Picchi, & Monteleone, 2010). Fresh bread samples were prepared on the same day as the test, allowed to cool at room temperature and then used for the sensory evaluation. The descriptive panel consisted of seven panellists (3 males and 4 females, age 20-40) familiar with cereal products. A training before the test was performed to define the sensory attributes (Table S1 in the supplementary material). A nine-point scale (1–9, from extremely weak to extremely strong, respectively) was used to rate intensity. The freshly baked bread samples were given three-digit codes and 2.5 cm slices were presented to the assessors in random order. Water was provided to cleanse the palate between the samples. The panel was instructed to smell each sample before tasting it, and then they were requested to swallow the samples. A qualitative evaluation was performed using the medians of the raw data obtained.

2.4.5 Data processing

Two replicates were carried out to in the T1 trial. A multi-factor ANOVA was performed to assess significant differences ($p < 0.05$) resulting from the seven tested factors.

In the T2 trial three replicates were carried out for dough rheology and four replicates for bread quality evaluation. A three-way ANOVA was performed to assess significant differences ($p < 0.05$) resulting from these factors and their two-factor and three-factor interactions. The Tukey HSD test was used as the post-hoc test.

3. Results and discussion

3.1 The T1 trial

Seven bread improvers were simultaneously tested on old wholewheat flour performance. Five of the seven improvers can be considered well-known bread improvers (i.e., EVOO, Suc, AH_2, GG and NaCl); GF and Ice, were also included.

GF from different sources has been tested in breadmaking (Carrillo-Navas et al., 2016; Fu, Che, Li, Wang, & Adhikari, 2016; Kim, Kwak, & Jeong, 2017). In particular, the addition of GF showed a significant improvement in the quality of the bread from brown wheat (Parenti et al., 2019).

The inclusion of Ice can be seen as a way to control a crucial factor of the kneading step: the temperature (Zhou et al., 2014). In preliminary trials different amounts of Ice (data not shown) in the breadmaking process were tested. The best result was obtained with a ratio of 20% (w/water w) of Ice: it reduced the dough temperature during the kneading step (20% of Ice addition reduced dough T before dough kneading from 20°C to 14°C and after dough kneading from 25°C to 20°C), without affecting this parameter during the leavening step and it gave the highest bread specific volume and softness.

The highest level of each factor was selected according to the literature as follows: 3%w/flour w EVOO (Pareyt, Finnie, Putseys, & Delcour, 2011), 2%w/flour w NaCl (Silow, Axel, Zannini, & Arendt, 2016); 6%w/flour w Suc (Zhou et al., 2014), 100ppm AH_2 (Tebben, Shen, & Li, 2018); 1%w/flour w GG (Tebben, Shen, & Li, 2018); 6% of the total flour added to the bread dough was used to prepare the GF (Parenti et al., 2019).

3.1.1 Rheological characteristics of old wholewheat flour and the dough samples

The farinographic values showed that the V1 batch of Verna old wholewheat flour was consistent with the "weak flour" definition: the reference consistency is reached quickly, to then decline considerably, with little or no stability (Zhou et al., 2014). Then, in "weak flours" an improvement in dough performance is usually related to an increase in dough stability (DS) and a reduction in dough weakening (DW). The alveographic values also showed a low value of dough strength (W) and an unbalanced ratio between dough tenacity and extensibility (P/L).

Addition of the improvers affected dough behaviour during the kneading step (Table 2). Except for the reduction of WA (approx. 2.9%), NaCl effect was consistent with the literature (Silow, Axel, Zannini, & Arendt, 2016): it strengthened the dough, increased DDT (approx. 1 min), triplicated DS and greatly reduced DW (approx. 90 DU). Similarly, GG significantly extended the DDT (more than 1 min), and increased DS (approx. 1 min). All these effects were consistent with previous studies (Tebben, Shen, & Li, 2018).

Considering the Suc effect, consistent with the literature (Peng, Li, Ding, & Yang, 2017) a decrease in WA, an increase in DS (approx. 1 min) and a decrease in DW (approx. 30 BU) were observed. Conversely, the decrease in DDT (approx. 1.5 min) was not in accordance with Mariotti & Alamprese (2012).

The addition of EVOO, GF and AH_2 did not result in an improvement in the farinographic performance. Specifically, EVOO decreased WA (2.6%) and slightly reduced DDT, without affecting DS or DW. The most common lipids used in breadmaking are shortening and surfactants, while very little investigation has been performed on the long chain fatty acids EVOO. The decrease in DDT could be the direct consequence of the lower amount of water required by dough with added EVOO.

GF significantly increased the WA parameter (2.9%), consistently with Parenti et al. (2019), and reduced the DDT (approx. 1 min), whereas no significant effects were observed on DS or DW.

Finally, AH_2 significantly decreased DDT and DS (approx. 1 min) without affecting the other parameters, worsening the old wholewheat flour's technological properties. These results were in contrast to the positive effect of an oxidant agents on white flours. It is likely that the fibre fraction of old wholewheat flour containing a high quantity of reducing compounds, lowered the effects of oxidant agents (Tebben, Shen, & Li, 2018).

Boosting dough rheological parameters thanks to NaCl, GG and Suc improvers could be seen as a good strategy to facilitate dough workability for the old wholewheat flour breadmaking process.

3.1.2 Bread quality

Fig. 1 compares the effects of the improvers on bread specific volume. An effect was observed for GF, EVOO, Suc and Ice, while the other improvers did not significantly affect the bread volume. Specifically, a significant increase was obtained with Suc (from 2.93 ± 0.08 L/kg to 3.15 ± 0.08 L/kg), EVOO (from 3.00 ± 0.08 L/kg to 3.09 ± 0.08 L/kg), and Ice (from 3.00 ± 0.08 L/kg to 3.09 ± 0.08 L/kg), while GF decreased the parameter from 3.19 ± 0.08 L/kg to 2.89 ± 0.08 L/kg.

The greatest rise in bread specific volume was obtained with Suc (7.4%), whereas EVOO and Ice produced a similar increase (3%). The effect of Suc probably promoted the growth of yeasts, which led to a better performance during the leavening step (Zhou et al, 2014).

The literature has reported no effect or a worsening effect on bread volume when vegetable oils are added to bread dough (Pareyt, Finnie, Putseys, & Delcour, 2011). Conversely, Matsakidou, Blekas, & Paraskevopoulou (2010), observed a significant volume increase when EVOO was added to cake dough production. The inclusion of Ice, which lowered the mixing temperature, could have improved the gluten matrix development (Quayson, Marti, Bonomi, Atwell, & Seetharaman, 2016). The negative effect of the GF, inconsistent with the literature (Parenti et al., 2019), could be the result of the different amylose/amylopectin ratio, which is a genetic characteristic of each wheat variety and deeply influences the starch gelatinization process (Goesaert et al., 2005).

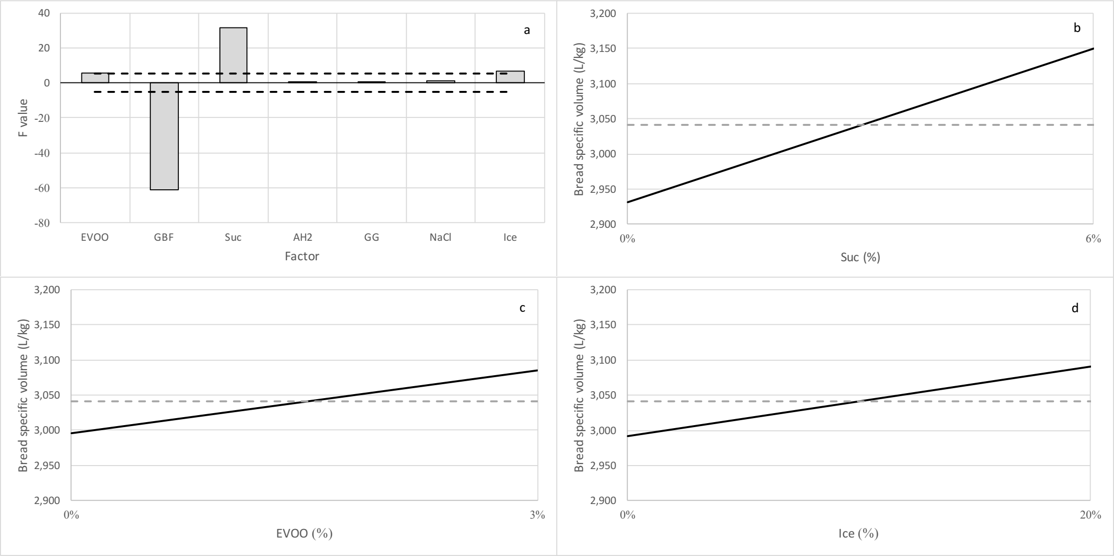

Fig. 1 Bar charts of T1 factors affecting bread specific volume (L/kg) (a). Line charts show the effect of addition of Suc (b), EVOO (c), and Ice (d) on bread specific volume (L/kg). Dashed line represents mean value of bread specific volume. The x-axis reports tested levels of each factor (Suc -1 = 0%, +1 = 6% w/flour w; EVOO -1 = 0%, +1 = 3% w/flour w; Ice -1 = 0%, +1 = 20% w/water w).

3.2 The T2 trial

This study aimed to optimize the bread quality, hence, only the improvers that positively affected the bread specific volume (i.e. Suc, EVOO and Ice) were selected for the T2 trial.

3.2.1 Rheological characteristics of old wholewheat flour and dough samples

According to the T1 trial, the farinographic test only considered Suc and EVOO as factors, while the addition of Ice was not tested. The V2 batch of Verna old wholewheat flour showed rheological properties consistent with the V1 batch.

All of the farinographic parameters were affected by Suc; EVOO significantly changed the WA, DS and DW. WA was significantly reduced by both factors (data not shown), in accordance with the T1 trial.

These results were consistent with the scientific literature; Peng, Li, Ding, & Yang (2017) reported a decrease in the WA parameter when a sugar (i.e. trehalose) was added to the bread dough; lipid improvers (i.e. shortening) decrease the flour components' adsorption capacity by settling around the starch granules and the gluten protein during the hydration phase (Pareyt, Finnie, Putseys, & Delcour, 2011).

The DDT was significantly enhanced by the addition of 4% Suc (from 2.8 ± 0.3 min to 3.1 ± 0.3 min): the greater the addition of the improver, the lower the water availability for the development of the gluten network, which requires a longer time (Mariotti & Alamprese, 2012).

DS was significantly improved by the highest level of Suc (from 2.1 ± 0.3 min to 2.4 ± 0.3 min) as well as by EVOO (from 2.0 ± 0.3 min to 2.5 ± 0.3 min). These results confirmed the effect of Suc already observed in the T1 trial. Furthermore, they revealed that EVOO exercised a comparable role. Finally, both improvers were effective in reducing DW: the highest level of Suc decreased the value from 177 ± 10BU to 166 ± 10BU, in accordance with the literature (Mariotti & Alamprese, 2012); a similar decrease was also observed with the inclusion of EVOO (from 177 ± 10BU to 164 ± 10BU). Hence, a general improvement of the rheological properties can be obtained by supplementing Suc and EVOO (Fig. 2). The positive effects exercised by Suc to the tested old wholewheat flour were consistent with those reported in the literature for conventional flour blends. Considering that there are few descriptions of the effects of EVOO in the literature, the results revealed it to be an improver of particular interest for old wholewheat flour rheological performance.

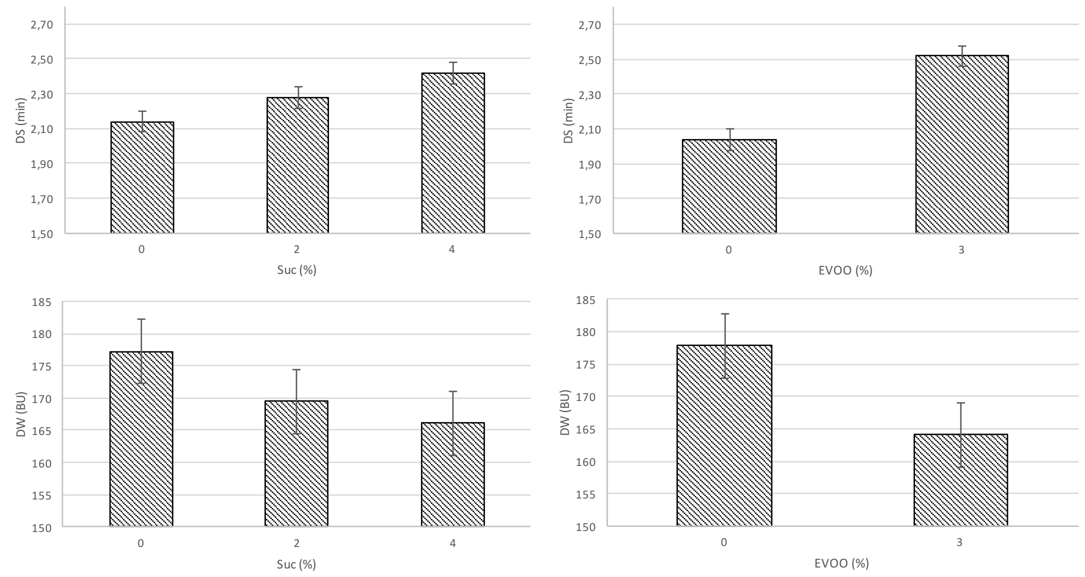

Fig. 2 Dough Stability (DS) and Dough Weakening (DW) farinographic parameters as affected by the addition of Suc (0%, 2%, 4% w/flour w) in a and c, and EVOO (0%, 3% w/flour w) in b and d.

Table 4. T2 bread quality evaluation

Sample	Suc (w/flour w)	EVOO (w/flour w)	Ice (w/water w)	Bread specific volume (L/kg)	Crumb specific volume (L/kg)	Crumb/dough moisture	Crust/dough moisture	Hardness (N)	Cohesiveness	Springiness (mm)	Chewiness (N mm)
1	0%	0%	0%	3.184 ± 0.097^{ax}	3.322 ± 0.252	96.9 ± 0.8^{ai}	67.4 ± 4.4^{x}	3.69 ± 0.73^{ax}	0.235 ± 0.058^{xi}	0.764 ± 0.066^{xi}	0.638 ± 0.256^{abxi}
2	0%	3%	0%	3.188 ± 0.097^{ay}	2.968 ± 0.252	96.9 ± 0.8^{ai}	55.8 ± 4.4^{y}	5.24 ± 0.73^{ay}	0.185 ± 0.058^{yi}	0.679 ± 0.066^{yi}	0.661 ± 0.256^{abyi}
3	0%	0%	10%	3.172 ± 0.097^{ax}	3.562 ± 0.252	97.6 ± 0.8^{aj}	66.3 ± 4.4^{x}	3.73 ± 0.73^{ax}	0.221 ± 0.058^{xi}	0.725 ± 0.066^{xij}	0.594 ± 0.256^{abxij}
4	0%	3%	10%	3.133 ± 0.097^{ay}	3.023 ± 0.252	97.4 ± 0.8^{aj}	62.0 ± 4.4^{y}	4.49 ± 0.73^{ay}	0.207 ± 0.058^{yi}	0.743 ± 0.066^{yij}	0.776 ± 0.256^{abyij}
5	0%	0%	20%	3.238 ± 0.097^{ax}	3.325 ± 0.252	97.5 ± 0.8^{ak}	70.5 ± 4.4^{x}	4.36 ± 0.73^{ax}	0.271 ± 0.058^{xj}	0.826 ± 0.066^{xj}	0.981 ± 0.256^{abxj}
6	0%	3%	20%	3.248 ± 0.097^{ay}	3.001 ± 0.252	97.4 ± 0.8^{ak}	59.5 ± 4.4^{y}	4.15 ± 0.73^{ay}	0.249 ± 0.058^{yj}	0.782 ± 0.066^{yj}	0.812 ± 0.256^{abyj}
7	2%	0%	0%	2.850 ± 0.097^{bx}	2.856 ± 0.252	97.3 ± 0.8^{abi}	66.6 ± 4.4^{x}	5.51 ± 0.73^{ax}	0.239 ± 0.058^{xi}	0.728 ± 0.066^{xi}	0.950 ± 0.256^{axi}
8	2%	3%	0%	3.215 ± 0.097^{by}	3.196 ± 0.252	98.2 ± 0.8^{abi}	61.6 ± 4.4^{y}	2.92 ± 0.73^{ay}	0.204 ± 0.058^{yi}	0.739 ± 0.066^{yi}	0.439 ± 0.256^{ayi}
9	2%	0%	10%	2.888 ± 0.097^{bx}	3.093 ± 0.252	98.6 ± 0.8^{abj}	68.5 ± 4.4^{x}	5.26 ± 0.73^{ax}	0.300 ± 0.058^{xi}	0.822 ± 0.066^{xij}	1.346 ± 0.256^{axij}
10	2%	3%	10%	3.149 ± 0.097^{by}	3.176 ± 0.252	98.5 ± 0.8^{abj}	66.2 ± 4.4^{y}	3.16 ± 0.73^{ay}	0.212 ± 0.058^{yi}	0.739 ± 0.066^{yij}	0.491 ± 0.256^{ayij}
11	2%	0%	20%	2.987 ± 0.097^{bx}	2.926 ± 0.252	98.8 ± 0.8^{abk}	71.4 ± 4.4^{x}	3.96 ± 0.73^{ax}	0.381 ± 0.058^{xj}	0.896 ± 0.066^{xj}	1.361 ± 0.256^{axj}
12	2%	3%	20%	3.048 ± 0.097^{by}	3.225 ± 0.252	98.2 ± 0.8^{abk}	62.2 ± 4.4^{y}	3.68 ± 0.73^{ay}	0.182 ± 0.058^{yj}	0.648 ± 0.066^{yj}	0.440 ± 0.256^{ayj}
13	4%	0%	0%	3.050 ± 0.097^{cx}	3.148 ± 0.252	98.1 ± 0.8^{bi}	67.8 ± 4.4^{x}	3.65 ± 0.73^{bx}	0.252 ± 0.058^{xi}	0.769 ± 0.066^{xi}	0.653 ± 0.256^{bxi}
14	4%	3%	0%	3.185 ± 0.097^{cy}	3.055 ± 0.252	98.0 ± 0.8^{bi}	64.5 ± 4.4^{y}	3.16 ± 0.73^{by}	0.144 ± 0.058^{yi}	0.655 ± 0.066^{yi}	0.279 ± 0.256^{byi}
15	4%	0%	10%	3.045 ± 0.097^{cx}	3.021 ± 0.252	98.9 ± 0.8^{bj}	72.2 ± 4.4^{x}	4.07 ± 0.73^{bx}	0.232 ± 0.058^{xi}	0.793 ± 0.066^{xij}	0.759 ± 0.256^{bxij}
16	4%	3%	10%	3.207 ± 0.097^{cy}	3.280 ± 0.252	98.5 ± 0.8^{bj}	61.1 ± 4.4^{y}	2.42 ± 0.73^{by}	0.198 ± 0.058^{yi}	0.754 ± 0.066^{yij}	0.355 ± 0.256^{byij}
17	4%	0%	20%	3.093 ± 0.097^{cx}	2.968 ± 0.252	98.9 ± 0.8^{bk}	69.6 ± 4.4^{x}	3.87 ± 0.73^{bx}	0.336 ± 0.058^{xj}	0.835 ± 0.066^{xj}	1.086 ± 0.256^{bxj}
18	4%	3%	20%	3.183 ± 0.097^{cy}	3.231 ± 0.252	98.2 ± 0.8^{bk}	61.5 ± 4.4^{y}	3.41 ± 0.73^{by}	0.195 ± 0.058^{yj}	0.689 ± 0.066^{yj}	0.446 ± 0.256^{byj}
p	Suc (a,b,c)			***	n.s.	***	n.s.	***	n.s.	n.s.	**
p	EVOO (x,y)			***	n.s.	n.s.	***	***	***	***	***
p	Ice (i,j,k)			n.s.	n.s.	*	n.s.	n.s.	**	*	**
p	Suc*EVOO			***	***	n.s.	n.s.	***	n.s.	n.s.	***
p	Suc*Ice			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
p	EVOO*Ice			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.
p	EVOO*Suc*Ice			n.s.	n.s.	n.s.	n.s.	** ?	n.s.	*?	n.s.

Data are expressed as mean ± standard deviations. Suc = sucrose, EVOO = extra virgin olive oil and Ice = ice. p Suc, p EVOO, p Ice, p Suc*EVOO, p Suc*Ice, p EVOO*Ice and p EVOO*Suc*Ice refer to main effects of Suc (p Suc), EVOO (p EVOO) and Ice (p Ice) factors and their two-factor (p Suc*EVOO, p Suc*Ice, p EVOO*Ice) and three-factor (p EVOO*Suc*Ice) interactions. *, ** and *** indicate significant differences at $p<0.05$, $p<0.01$ and $p<0.001$, respectively; "n.s." indicates no significant difference at $p<0.05$. Means in a column

3.2.2 Bread quality

The experimental data of the bread quality characteristics are shown in Table 4.

Considering bread specific volume, the Suc*EVOO interaction had a significant effect (Fig. 3). Specifically, the above parameter was optimized by EVOO, since regardless of Suc levels, the value increased by approx. 11%. This effect was not consistent with the literature on vegetable oils; furthermore, the presence of solid ß’ crystals in the shortening seemed crucial for the stabilization of gas bubbles and the increase in bread volume (Pareyt, Finnie, Putseys, & Delcour, 2011). However, the literature also reports that different lipid typologies show very different effects (Autio & Laurikainen, 1997). Considering the unique chemical composition of EVOO, different effects may be associated with this improver, as shown by Matsakidou, Blekas, & Paraskevopoulou (2010).

The highest level of Suc significantly increased bread specific volume (7%). This result was probably linked to the well-known effects of Suc on the breadmaking process: (i) an increase in starch gelatinization temperature, resulting in a higher crumb porosity (Psimouli & Oreopoulou, 2012), (ii) higher fermentative activity with a rise in CO_2 production and (iii) a greater increase in the volume of the final product (Zhou et al., 2014).

The Suc*EVOO interaction had a significant effect on the crumb specific volume. In contrast with the bread specific volume, the inclusion of Suc as a single improver reduced the parameter. The addition of EVOO together with Suc, regardless of the level of Suc, gave the best result, increasing the crumb specific volume (Fig. 3). Probably, a synergic effect between the two improvers occurred.

Looking at the moisture parameters, Suc and Ice slightly but significantly increased the crumb moisture (1%), whereas EVOO significantly reduced the crust moisture, lowering the value by around 10%.

All the improvers had a significant effect in the TPA analysis. The hardness was significantly affected by the Suc*EVOO interaction (Fig. 3). The parameter was optimized with both Suc and EVOO, which reduced the value by about 17-20%. Considering cohesiveness, EVOO and Ice had a significant effect. Specifically, EVOO significantly reduced the parameter, while Ice determined a significant increase. Since cohesiveness is inversely related to water content, these results are consistent with the amount of water in the sample; indeed, the addition of EVOO significantly lowered the dough water requirement (WA), while Ice significantly increased crumb moisture. With regard to springiness, the EVOO*Ice interaction had a significant effect: without EVOO addition, the highest level of Ice boosted springiness by about 24%. Chewiness was significantly affected by EVOO*Suc and Suc*Ice interactions. The best value, the lowest one according to the literature (Peng, Li, Ding, & Yang, 2017), was achieved by adding EVOO and the highest level of Suc (50.6%). Interestingly, the best improvement in chewiness was achieved with the combination of Suc and EVOO, as already observed on the specific volume parameters (Fig. 3). The Suc*Ice interaction showed that the highest level of Ice only combined with the highest level of Suc increased chewiness (58%), hence reducing the product quality.

Table 5 reports the experimental data on bread image and bread colour analysis. Considering the median pore area, the EVOO*Ice and Suc*Ice interactions exercised a significant effect. In detail, the highest level of Ice significantly reduced the parameter when combined with EVOO as compared to the value observed without the addition of EVOO. The second interaction showed that the highest level of Ice increased the pore area when Ice was the sole improver added. The addition of EVOO reduced the ratio between pore area/total pore area, revealing a similar effect to that of shortening in decreasing the pore size and probably improving crumb evenness (Pareyt, Finnie, Putseys, & Delcour, 2011).

Concerning colour analysis, all of the bread samples displayed an acceptable both crust and crumb colour. The crumb colour results outlined a significant increase in the L* parameter, as a consequence of the highest level of Suc (4.2%). All parameters related to crust colour were significantly affected by Suc and EVOO. Specifically, L* was reduced by Suc (6.4%), and increased by EVOO (5.2%). Moreover, the a* parameter was increased by Suc (62.3%), while EVOO lowered it (19.1%). Finally, the b* parameter showed a similar trend to a*: an increase with Suc (15.2%) and a reduction with EVOO (6.7%).

Hence, this analysis revealed that Suc had a significant effect: it enhanced crumb brightness, reduced crust brightness and increased its yellow and red components. However, only the highest level of Suc exercised a significant effect on bread colour, probably because the lower level was entirely depleted by yeasts during fermentation, without leaving any reducing sugars in the final dough for non-enzymatic browning reactions. The addition of EVOO significantly affected crust colour, too; it increased crust brightness as well as reduced the red and yellow components.

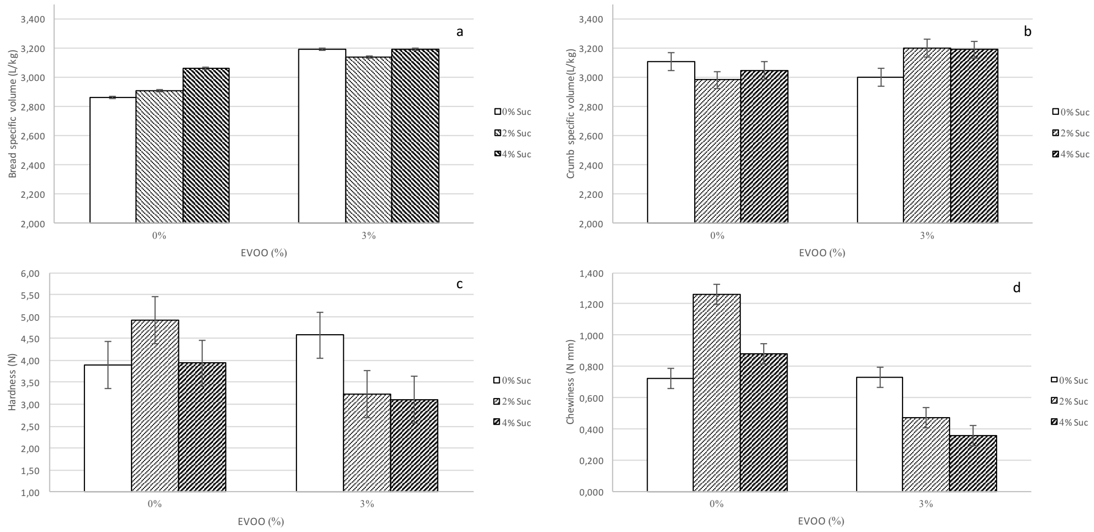

Fig. 3 Effects of Suc*EVOO interaction on: a) bread specific volume (L/kg), b) crumb specific volume (L/kg), c) hardness (N) and d) chewiness (Nmm).

Table 5. T2 trials bread quality evaluation.

Sample	Suc (w/flour w)	EVOO (w/flour w)	Ice (w/water w)	Pore area 0.5 (mm^{2})	Pore area/area tot (%)	Crumb L*	Crumb a*	Crumb b*	Crust L*	Crust a*	Crust b*
1	0%	0%	0%	2.71 ± 0.35^{x}	29.55 ± 2.88^{x}	60.00 ± 2.97^{a}	4.75 ± 0.96	15.50 ± 3.79	52.00 ± 2.29^{ax}	9.75 ± 1.80^{ax}	30.25 ± 3.10^{ax}
2	0%	3%	0%	2.88 ± 0.35^{y}	27.33 ± 2.88^{y}	63.75 ± 2.97^{a}	4.00 ± 0.96	14.75 ± 3.79	55.00 ± 2.29^{ay}	6.50 ± 1.80^{ay}	27.50 ± 3.10^{ay}
3	0%	0%	10%	3.05 ± 0.35^{x}	29.55 ± 2.88^{x}	59.75 ± 2.97^{a}	3.50 ± 0.96	12.75 ± 3.79	50.25 ± 2.29^{ax}	9.00 ± 1.80^{ax}	29.50 ± 3.10^{ax}
4	0%	3%	10%	2.91 ± 0.35^{y}	29.74 ± 2.88^{y}	59.75 ± 2.97^{a}	4.00 ± 0.96	16.25 ± 3.79	55.50 ± 2.29^{ay}	7.00 ± 1.80^{ay}	27.75 ± 3.10^{ay}
5	0%	0%	20%	3.76 ± 0.35^{x}	28.39 ± 2.88^{x}	58.13 ± 2.97^{a}	4.13 ± 0.96	13.50 ± 3.79	51.75 ± 2.29^{ax}	8.63 ± 1.80^{ax}	29.13 ± 3.10^{ax}
6	0%	3%	20%	2.98 ± 0.35^{y}	26.23 ± 2.88^{y}	64.00 ± 2.97^{a}	3.25 ± 0.96	13.50 ± 3.79	56.25 ± 2.29^{ay}	5.50 ± 1.80^{ay}	27.25 ± 3.10^{ay}
7	2%	0%	0%	3.04 ± 0.35^{x}	28.28 ± 2.88^{x}	63.50 ± 2.97^{ab}	3.50 ± 0.96	14.25 ± 3.79	50.50 ± 2.29^{ax}	9.50 ± 1.80^{ax}	31.00 ± 3.10^{ax}
8	2%	3%	0%	2.98 ± 0.35^{y}	26.60 ± 2.88^{y}	62.25 ± 2.97^{ab}	3.50 ± 0.96	13.25 ± 3.79	54.40 ± 2.29^{ay}	7.00 ± 1.80^{ay}	27.00 ± 3.10^{ay}
9	2%	0%	10%	2.95 ± 0.35^{x}	28.36 ± 2.88^{x}	61.75 ± 2.97^{ab}	4.00 ± 0.96	14.50 ± 3.79	51.50 ± 2.29^{ax}	9.75 ± 1.80^{ax}	31.75 ± 3.10^{ax}
10	2%	3%	10%	2.80 ± 0.35^{y}	24.91 ± 2.88^{y}	61.75 ± 2.97^{ab}	3.75 ± 0.96	16.75 ± 3.79	52.25 ± 2.29^{ay}	7.75 ± 1.80^{ay}	27.25 ± 3.10^{ay}
11	2%	0%	20%	3.20 ± 0.35^{x}	30.18 ± 2.88^{x}	61.75 ± 2.97^{ab}	3.25 ± 0.96	14.25 ± 3.79	52.00 ± 2.29^{ax}	9.50 ± 1.80^{ax}	30.50 ± 3.10^{ax}
12	2%	3%	20%	2.60 ± 0.35^{y}	26.97 ± 2.88^{y}	62.50 ± 2.97^{ab}	3.50 ± 0.96	15.00 ± 3.79	53.75 ± 2.29^{ay}	8.75 ± 1.80^{ay}	32.00 ± 3.10^{ay}
13	4%	0%	0%	3.15 ± 0.35^{x}	29.03 ± 2.88^{x}	65.50 ± 2.97^{b}	4.00 ± 0.96	15.25 ± 3.79	50.75 ± 2.29^{bx}	13.00 ± 1.80^{bx}	34.00 ± 3.10^{bx}
14	4%	3%	0%	2.91 ± 0.35^{y}	28.77 ± 2.88^{y}	63.75 ± 2.97^{b}	3.50 ± 0.96	17.00 ± 3.79	52.00 ± 2.29^{by}	9.75 ± 1.80^{by}	29.00 ± 3.10^{by}
15	4%	0%	10%	3.09 ± 0.35^{x}	30.96 ± 2.88^{x}	61.25 ± 2.97^{b}	3.75 ± 0.96	16.25 ± 3.79	48.00 ± 2.29^{bx}	14.25 ± 1.80^{bx}	33.50 ± 3.10^{bx}
16	4%	3%	10%	3.19 ± 0.35^{y}	27.74 ± 2.88^{y}	62.75 ± 2.97^{b}	3.50 ± 0.96	14.25 ± 3.79	50.25 ± 2.29^{by}	12.75 ± 1.80^{by}	33.75 ± 3.10^{by}
17	4%	0%	20%	2.95 ± 0.35^{x}	30.93 ± 2.88^{x}	64.25 ± 2.97^{b}	3.75 ± 0.96	13.50 ± 3.79	49.25 ± 2.29^{bx}	12.75 ± 1.80^{bx}	34.00 ± 3.10^{bx}
18	4%	3%	20%	2.74 ± 0.35^{y}	28.68 ± 2.88^{y}	63.25 ± 2.97^{b}	3.75 ± 0.96	16.50 ± 3.79	50.00 ± 2.29^{by}	12.75 ± 1.80^{by}	33.25 ± 3.10^{by}
p	Suc (a,b,c)			n.s.	n.s.	*	n.s.	n.s.	***	***.	***
p	EVOO (x,y)			*	**	n.s.	n.s.	n.s.	***	***	**
p	Ice (i,j,k)			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
p	Suc*EVOO			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
p	Suc*Ice			*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
p	EVOO*Ice			*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
p	EVOO*Suc*Ice			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Data are expressed as mean ± standard deviations. Suc = sucrose, EVOO = extra virgin olive oil and Ice = Ice. p Suc, p EVOO and p Ice refer to the main effects of these factors; p Suc*EVOO, p Suc*Ice and p EVOO*Ice refer to the effect of the two-factor interactions; p EVOO*Suc*Ice Suc refers to three-factor interaction. *, ** and *** indicate significant differences at $p<0.05$, $p<0.01$ and $p<0.001$, respectively; "n.s." indicates no significant difference at $p<0.05$. Means in a column with different superscripts are significantly different ($p<0.05$). Specifically, "a", "b" and "c" refer to main effect of Suc, "x" and "y" refer to main effect of EVOO and "i", "j" and "k" refer to main effect of Ice.

3.2.3 Optimization of bread ingredients and bread sensory evaluation

The results of the T2 trial were analysed with the aim of optimizing bread quality. Bread specific volume, crumb specific volume and bread hardness were considered the most representative parameters of product quality. The bread specific volume was maximized with EVOO, while for the optimization of the crumb specific volume and hardness, the combination of Suc and EVOO was required. Indeed, the highest crumb specific volume and the lowest hardness was obtained with Suc 2% and EVOO. No significant difference was obtained when the Suc was increased from 2% and 4%.

Since the aim of the study was to combine the optimization of technological properties with the preservation of the nutritional value of old wholewheat flour, the choice was to minimize the addition of improvers. Hence, Suc at 2% and EVOO at 3% were chosen for the optimized recipe.

The optimized sample was subjected to a qualitative sensory evaluation in comparison to the control sample (i.e. without improvers). Fig. 4 outlines the bread slice, bread crumb and bread crust results. The panel perceived differences for all the bread portions analysed. For the bread slices, the attributes that most discriminated the two samples were acidulous and cereal aromas, both perceived as more intense in the optimized bread. The bread crumb revealed the greatest differences in the following attributes: elasticity, moisture, solubility, brewer’s yeast flavour and sourness. All these attributes except elasticity resulted more intense in the optimized sample than in the control. Considering the crust evaluation, the greatest differences were perceived in the friability, saltiness and brewer’s yeast flavour, which received a higher score for the optimized bread.

The highest intensity of acidulous aroma, sourness and brewer’s yeast flavour could be linked to the inclusion of Suc, which probably increased the yeast growth and metabolic activity (Zhou et al., 2014).

The solubility descriptor of bread crumb was perceived as higher, in accordance with the TPA results, which showed the lowest hardness value. The highest value for the crumb moisture attribute is consistent with the physical parameter, which revealed an increase of 1%. The bread crust of the optimized sample, perceived as more friable, could be the result of its lower moisture content (10%). This moisture difference may also have emphasized the taste of the crust, making it seem saltier: the lower the water content, the higher the solute concentration. Finally, the elasticity value proved to be lower than the control sample, consistently with the TPA analysis.

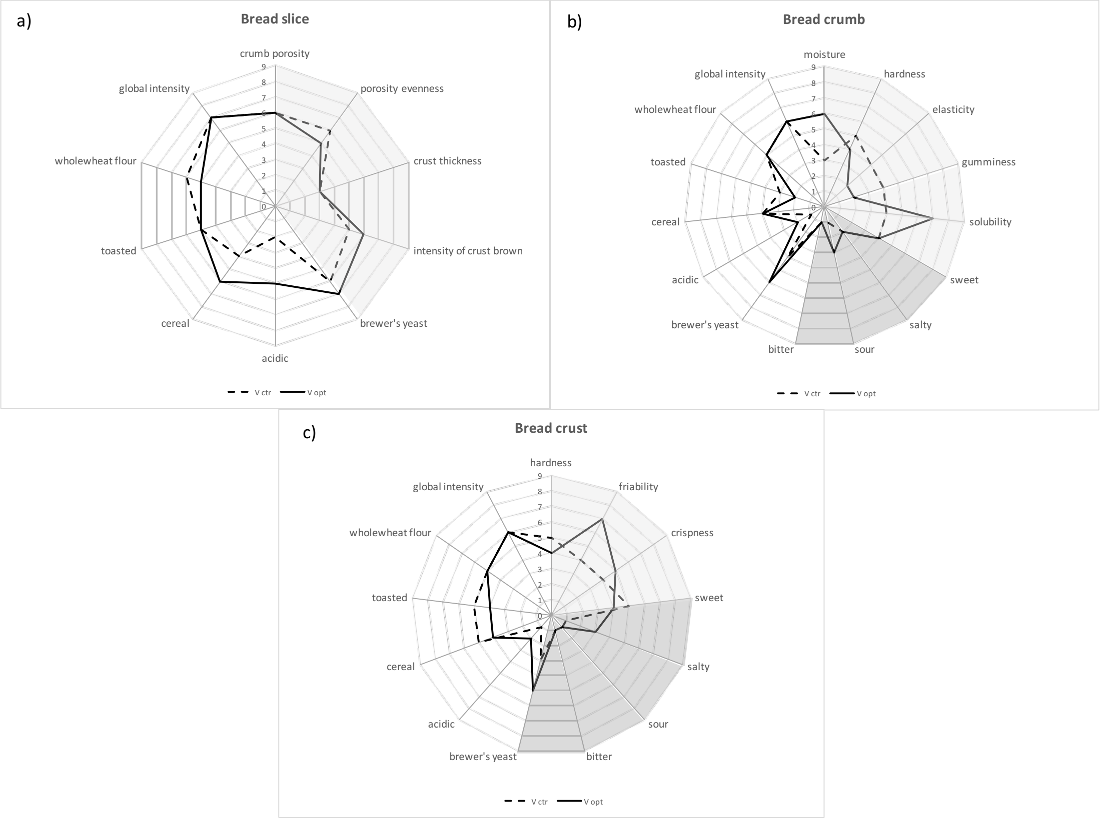

Fig. 4 Sensory evaluation of bread slices (a), bread crumb (b) and bread crust (c). Sectors with different colours correspond to different classes of descriptors: aroma (white) and appearance (light grey) descriptors for bread slices; touch (light grey), taste (grey) and flavour (white) descriptors for bread crumb and crust. Reported values are medians of the raw data.

4. Conclusions

Old wholewheat flours are characterized by an interesting nutritional profile, but they showed a very poor technological performance. Hence, the use of old wholewheat flour for the breadmaking process requires appropriate techniques, specifically designed for the different characteristics of the raw material compared to conventional flours.

By applying a two-step experiment (a screening step and a validation step), we selected the optimal combination of flour improvers to increase the bread quality. Suc (2%) and EVOO (3%) were identified as the optimized mixture of ingredients to improve bread quality.

The possibility of adopting this optimization method with other old wholewheat flours may be an interesting tool to design old wholewheat flour breadmaking. Indeed, if the breadmaking process is designed to optimize the specific characteristics of bread, an improvement could be obtained in product quality. Thereby, the use of old wholewheat flour in the bakery industry could be increased, promoting the consumption of healthier breads as well as safeguarding Triticum genus biodiversity.

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4.4 Molecular insight of unrefined dough kneading

4.4.1 Preliminary remark – Article 5

After having focused on the optimization of the bread formula of unrefined wheat flours (Parenti et al., 2019; Parenti et al. 2020b), the Doctoral research moved on the investigation of strategies able to adapt the processing conditions to the specific requirements of unrefined wheat flours. Indeed, several scientific evidences showed that the presence of different amounts of milling by-products negatively affect the bread-making performance of wheat flours resulting in poor loaf volume, dark colour, dense and firm texture and bitter taste (Hemdane et al., 2016; Boukid et al., 2018). Similarily to the discussion previously made on bread improvers, since the standardization of the bread-making process has been designed to optimize the performance of refined flours, different conditions may be necessary when refined flours are substituted with unrefined flours. One of the most critical phase of the entire bread-making process is the kneading operation; hence, we decided to focus the thesis research on this phase of the process.

During kneading, several physical-chemical phenomena occur within the dough giving a characteristic visco-elastic dough as a result. The analysis of the literature revealed scant information concerning the investigation of physical-chemical phenomena at a molecular scale that the constituents of wheat flour undergone during the kneading operation. In recent years, ^{1}H NMR spectroscopy has been shown to be a powerful molecular technique with interesting applications in food science (Hatzakis, 2019). Several studies on cereal based products have shown that this technique is able to analyse the biopolymer physical-chemical transformations and water redistribution during the bread-making process. This technique has been successfully applied to investigate the molecular phenomena occurring in the different phases of the bread-making process, particularly in heating and cooling conditions and during bread storage (Curti et al., 2013; Curti et al., 2015; Bosmans et al., 2016). However, scant information has been reported in the literature on the proton molecular dynamics and mobility during the kneading operation of bread dough.

These considerations triggered the experimental research of the Doctoral thesis on the application of ^{1}H NMR technique to describe the kneading step of unrefined flour dough. The effect of the both the kneading time and water amount was investigated. The study allowed to obtain information about the main proton poplations that characterised unrefined wheat flour dough, which were assigned to specific wheat flour domains according to the literature. Furthermore, the evolutions of the relative abundance of these proton populations and their relaxation times during kneading were analysed in order to gain a molecular insight into the physical-chemical modifications of flour biopolymers as well as the water redistribution among the main flour constituents.

4.4.2 Article 5

Parenti, O., Guerrini, L., Zanoni, B., Marchini, M., Grazia, M., & Carini, E. (2021). Use of the ^{1}H NMR technique to describe the kneading step of wholewheat dough: The effect of kneading time and total water content. *Food Chemistry*, 338, 128120. https://doi.org/10.1016/j.foodchem.2020.128120

Use of the ^{1}H NMR technique to describe the kneading step of wholewheat dough: the effect of kneading time and total water content

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Abstract

The kneading step of wholewheat flour (WWF) dough was monitored using low-resolution ^{1}H nuclear magnetic resonance (NMR). The tested variables were kneading time and total water content. Two ^{1}H Free induction decay (FID) (A and B) and four ^{1}H T_2 Car-Purcell-Meiboom-Gill (CPMG) (C, D, E and F) proton populations were observed and the attribution to the different proton domains was made based on the literature and data acquisition. Kneading time significantly increased the mobility and the relative abundance of popA, the relative abundance and strength of protons of popC, D and E, while significantly reducing the relative amount of popF and increasing its mobility. This evolution of the proton populations during kneading was interpreted as chemical/physical transformations of the flour constituents. The use of WWF may reveal the changes in molecular dynamics underlying the higher water requirements of unrefined doughs, often associated with improved bread quality.

Keywords

unrefined flour, mixing step, wheat dough, molecular mobility

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1. Introduction

Low-resolution (LR) proton nuclear magnetic resonance (^{1}H NMR) is a powerful, non-destructive technique that is used to evaluate food quality due to its ability to study the molecular mobility and dynamics of water and biopolymers during the processing and storage of foods (Kirtil, Cikrikci, Mccarthy, & Oztop, 2017).

In the literature, LR ^{1}H NMR analysis has been widely applied to cereal-based products, particularly to investigate the chemical and physical status of flour biopolymers and their interactions with water molecules in dough, bread, and flour model systems (Bosmans & Delcour, 2016). Studies on flour polymers have investigated ^{1}H NMR distributions of relaxation times in model systems, while trying to assign the different sample proton populations to protons of the main flour constituents, i.e., starch and gluten, and to water protons (Tang, Godward, & Hills, 2000; Tang, Brun, & Hills, 2001; Choi, & Kerr, 2003; Doona, & Baik, 2007; Bosmans, Lagrain, Deleu, Fierens, Hills, & Delcour, 2012). Some authors have applied the LR ^{1}H NMR technique to study wheat flour dough with different water contents and during simulated breadmaking conditions. These experiments have highlighted the molecular water dynamics and redistribution among biopolymers, as well as the physico-chemical transformations experienced by the biopolymers in wheat dough during heating and cooling, and during bread staling (Ruan, Wang, Chen, Fulcher, Pesheck, & Chakrabarti,1999; Kim, & Cornillon, 2001; Lopes Da Silva, Santos, Freitas, Brites, & Gil, 2007; Doona, & Baik, 2007; Lu, & Seetharaman, 2013; Rondeau-Mouro, Cambert, Kovrlija, Musse, Lucas, & Mariette, 2015; Nivelle, Beghin, Bosmans, & Delcour, 2019; Hopkins, Newling, Hucl, Scanlon, & Nickerson, 2019; Curti, Carini, Cobo, Bocher, & Vittadini, 2017). In this light, ^{1}H NMR has been also used to investigate the effect of the incorporation of milling by-products (bran and germ) on the proton mobility of both wheat doughs (Adams, Ragaee, & Abdel-aal, 2016; Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin, 2017a,b; Li, Hou, Chen, Chung, & Gehring, 2014; Li, Liu, Wu, Wang, & Zhang, 2016; Lu, & Seetharaman, 2013; Wang, Ye, Li, Wei, Chen, & Zhao, 2017; Xiong, Zhang, Niu, & Zhao, 2017) and fresh and stored bread (Katina, Salmenkallio-Marttila, Partanen, Forssell, & Autio, 2006; Curti, Carini, Bonacini, Tribuzio, & Vittadini, 2013; Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin, 2017b).

However, despite the molecular insights on wholewheat dough and bread, and the correlations with their macroscopic properties shown in the literature, at the present time the use of unrefined flours in the breadmaking process is still an issue (Parenti, Guerrini, & Zanoni, 2020).

It is widely known that kneading is one of the most important phases in breadmaking. This stage enables the homogeneous mixing of all the ingredients, the hydration of the flour constituents, the phase transitions that involve proteins and amorphous starch, the development of the gluten network, and the inclusion of air bubbles, giving a viscoelastic dough as a result (Cuq, Yildiz, & Kokini, 2002; Zhou, Therdthai, & Hui, 2014). Kneading conditions significantly affect dough development and its rheological properties, the breadmaking performance and the quality of the final product (Zhou, Therdthai, & Hui, 2014); furthermore, flours with different degrees of refinement may require adapted kneading conditions and higher amounts of water than refined flours (Cappelli, Cini, Guerrini, Masella, Angeloni, & Parenti, 2019). Indeed, it is well known that the presence of the fibre fraction significantly changes the water redistribution during the entire breadmaking process. In the kneading step, competition for the water molecules may occur between the flour constituents and the fibre, which could negatively affect the gluten network formation (Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin, 2017b).

To the best of the authors' knowledge, in the current literature there are very few studies that have applied the LR ^{1}H NMR technique to monitor proton mobility in wheat dough during the first step of the breadmaking process, i.e., kneading (Kim, & Cornillon, 2001; Sangpring, Fukuoka, Ban, Oishi, & Sakai, 2017). This research includes the study by Kim, & Cornillon (2001) who studied the molecular mobility of wheat doughs at the end of different kneading periods (3, 18 and 30 min) and during a heating treatment (from 30°C to 100°C). Furthermore, the work by Sangpring, Fukuoka, Ban, Oishi, & Sakai (2017) investigated the relationship between the mixing state of wheat flour dough and the mechanical energy generated using a vertical mixer, testing different revolution speeds for a total kneading time of 3 min (Sangpring, Fukuoka, Ban, Oishi, & Sakai, 2017).

In the present study, the ^{1}H NMR technique was applied to monitor the proton molecular dynamics and mobility in wholewheat flour (WWF) dough during the kneading step. Furthermore, due to the key role of the water amount in WWF dough, a comparison was made between the proton distributions obtained at two different dough moisture contents.

A single pulse free induction decay (FID) and Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (^{1}H T_2 spin-spin relaxation) were applied to measure both fast relaxing and slowly relaxing protons. The kinetic evolution of the mobility and abundance of ^{1}H populations were monitored during the process so as to gain a new insight into the kneading phenomenon. The choice to use a WWF could improve the understanding of the molecular phenomena linked to the presence of milling by-products and could disclose new strategies for the development of processing conditions adapted in function of the characteristics of the raw material.

2. Materials and Methods

2.1 Materials

One batch of sp. *Triticum aestivum* L., cv. Verna WWF, was used to perform the experimental trial. The wheat was grown in Montespertoli (Florence, Italy) during the 2019-2020 growing season.

The WWF was ground using a stone grinding mill and a sieve (two consecutive passages through a 1,100-1,200 μm sieve) at the Molino Paciscopi (Montespertoli, Florence, Italy). The flour belongs to the wholewheat category according to the Italian classification as the extraction rate and the ash content were in line with the standard benchmarks for this flour category (i.e., extraction rate 98 g/100 g dry kernel, ash content 1.3-1.7 g/100 g dm) (Zhou, Therdthai, & Hui, 2014). The mineral water (Sant'Anna, Vinadio, Italy) was purchased at a local market (Florence, Italy).

2.2 The experimental design

The ^{1}H NMR molecular mobility of WWF dough was studied using a full factorial design. The experimental trial tested the effect of two variables:

(i) The kneading time, i.e., *t*. Measurements were performed after every 3 min of kneading, from 3 to 24 min for a total of 8 measurements (t_1=3 min, t_2=6 min, t_3=9 min, t_4=12 min, t_5=15 min, t_6=18 min, t_7=21 min and t_8=24 min);

(ii) The total water content, i.e., *TW*, (%, g water/100 g flour). Two different levels were tested: 56% (w/flour w), i.e., the WWF56 samples, which corresponded to the amount of water to achieve a farinographic consistency of 500BU, vs 60% (w/flour w), i.e., the WWF60 samples.

2.3 Measurement methods

2.3.1 ^{1}H NMR measurements

Proton molecular mobility was investigated with a low-resolution (20 MHz) ^{1}H NMR spectrometer (the MiniSpec, Bruker Biospin, Milano, Italy) operating at 25.0 ± 0.1°C. ^{1}H free induction decay (FID) and ^{1}H T_2 Carr-Purcell-Meiboom-Gill (CPMG) experiments were used. The FID experiment allows to detect very short relaxation time (in the range between 10-500 μs) which correspond to the less mobile protons in solid-like components and of protons of water molecules tightly associated with those of solids. Conversely, the more mobile protons (in the range between 0.1- 1000 ms) has to be detected with CMG pulse-sequence as the high relaxation times measured using FID sequence are not true spin–spin relaxation times because the FID signal contains also the lost signal due to local inhomogeneities in the magnetic field.

The dough ingredients were stored at room temperature (22 ± 2°C) and 500 g batches of dough were prepared; the basic formulation was: flour (310 g) and water (56% and 60% w/flour w). The dough was prepared at room temperature (22 ± 2°C) using a Kitchen Aid Professional Mixer (5KSM185PS, KitchenAid, St. Joseph, Michigan, USA) with a dough hook (model KSM35CDH), functioning at 110 rpm. Samples were analysed every 3 min during the kneading step for a total of 8 kneading periods (see above).

Due to the time required for the acquisition of the ^{1}H NMR signals and to the short time interval between the selected kneading points (3 min), and to ensure that all samples were analysed within a maximum of 1 minute after kneading (to avoid different resting times), two batches of dough had to be prepared for each replicate. Specifically, in order to be able to measure the 8 kneading times, the same dough replicate required the analysis of two different batches: in the first batch, the ^{1}H NMR parameters were acquired from each acquisition (t_3, t_9, t_{15}, t_{21}) after a 6-min interval; in the second batch, the complementary kneading points were analysed at the same time interval (t_6, t_{12}, t_{18}, t_{24}) in order to complete the ^{1}H NMR molecular kinetic of the dough. Therefore, variability was inevitably introduced to the data set due to the different dough mixing batches, since each dough replicate did not derive from the same sample and, as it is a complex food matrix, bread dough is known to have an intrinsically high level of variability. Hence, the experiments required the preparation of a total of 2 (dough samples) x 4 (replicates) x 2 (water levels) = 16 batches of dough.

Dough samples (approx. 4 g) were collected from the central part of the dough during kneading. These were quickly placed in 10 mm diameter NMR tubes, and tightly compressed to a height of 10.5 mm. The tubes were then sealed with Parafilm to prevent moisture loss during the experiment.

FIDs signals were acquired using a single 90° pulse, followed by a dwell time of 7 μs and a recycle delay of 1 s, a 0.5 ms acquisition window (the experimental window limit for ensuring the homogeneity of the magnetic field), 32 scans and 900 data points. Six ^{1}H FID replicates were acquired for each sample. A two-component (exponential and Gaussian) model was used to fit the curves in order to obtain quantitative information about the proton relaxation time and the percentage of protons belonging to the more rigid and more mobile proton populations measurable within the FID experimental time frame (7–500 μs). The FID curves were fitted using SigmaPlot v.6 software (Systat Software Inc., USA), according to the following equation:

$$f(x) = y0 + ae^{\left(-\frac{t}{TA}\right)} + ce^{\left(-\frac{t}{TB}\right)^2} \qquad [1]$$

where *y*0 is the intercept, *a* and *c* the relative abundance of populations A and B, and T_A and T_B the relaxation time of the relative populations.

^{1}H T_2 (transverse relaxation time) was obtained with a CPMG pulse sequence with a recycle delay of 1 s, an interpulse spacing of 0.04 ms, 2500 data points and 32 scans. In order to increase the signal-to-noise ratio, a high number of scans were applied. A high number of scans increases the temperature of the sample and a temperature equilibrium period is generally required before the next experiment. In this study it was not possible to wait an additional amount of time, as the dough resting time could have affected the ^{1}H T_2 signal. Thus, only one ^{1}H T_2 curve was acquired for each dough replicate, for a total of at least four replicates for each sample. This aspect strongly underlines the great capability of the experimental plane to represent ^{1}H dynamics and mobility in such a complex matrix as bread dough during kneading time. The ^{1}H T_2 curves were analysed as quasi-continuous distributions of relaxation times using UPEN software (Alma Mater Studiorum, Bologna, Italy). ^{1}H T_2 CPMG relaxation decays were also fitted with a discrete exponential model (Sigmaplot, v.6, Systat Software Inc., USA) in order to obtain relaxation times and proton population abundances, according to the following equation:

$$f(x) = y0 + ae^{-bx} + ce^{-dx} + ge^{-hx} + ie^{-fx} \qquad [2]$$

where $y0$ is the intercept, a, c, g and i the relative abundance of populations C, D, E and F, and b, d, h and f the relaxation time of populations C, D, E and F.

2.4 Modelling and data processing

In order to predict the ^{1}H NMR parameters of the different water content dough samples (56% vs 60%) during kneading, the experimental data were fitted with a linear model for the continuous t variable and the categorical TW variable, and with a second-order model for the continuous variable t, according to the following equation:

$$y_{obs} = b_0 + b_1 t + b_2 TW + b_{12} tTW + b_{11} t^2 + b_{112} t^2 TW + \text{error} \qquad [3]$$

where b_0 is a constant (the intercept); b_1 and b_2 represent the main effect of each factor (t and TW); b_{12} is the effect of the interaction between the first-order coefficient of the variables ($t*TW$); the square coefficient b_{11} reveals if the variable t gives a maximum or minimum within the experimental domain; and b_{112} represents the effect of the interaction between the second-order coefficient of t and TW (t^2*TW).

The data were analysed with R software. A two-way ANOVA was performed in order to assess significant differences ($p < 0.05$) due to the tested variables (t and TW) and to their interaction ($t*TW$). The not significant terms ($p > 0.05$) were removed from the model as suggested by Dunn, & Smyth (2018). Following this, the model was further checked with the ANOVA model.

3. Results

3.1 ^{1}H NMR proton distributions

The FID experiment showed the presence of two proton populations which were named A (the less mobile ones) and B (the more mobile ones), relaxing in the range of 15.2-15.7 μs and 349.4-368.8 μs, respectively. The ^{1}H T_2 distributions of the relaxation times showed the presence of four populations identified as popC, popD, popE and popF, from the least to the most mobile proton population, respectively. The ^{1}H relaxation times were in the range of 0.29-0.50 ms, 3.25-4.04 ms, 10.03-15.03 ms and 41.84-53.76 ms for populations C, D, E and F, respectively. Considering the abundances of the ^{1}H populations, the relative abundance of population A + population B gives 100% of the FID proton signal, while the relative abundance of populations C, D, E and F gives 100% of the CPMG signal. The dominant FID population was population A, which encompassed 78.23-80.17% of the total observable protons (population B represented 19.83-21.77% of the total protons). In the ^{1}H T_2 time frame window, the dominant population was population E, representing 52.57-56.79% of the total detectable protons, followed by population D (25.56-29.91%), population C (8.26-10.49%) and population F (5.40-14.60%). Since the relaxation times of populations B and C overlapped, these proton populations were considered to represent the same protons and therefore only population C was discussed as belonging to the better resolved CPMG experiment signal. As further confirmation of this hypothesis, the relaxation time of populations B and C showed the same results in function of the tested variables.

3.2 The effect of the kneading time

Kneading time, i.e., the variable *t*, significantly affected the ^{1}H NMR distributions. Considering the FID signal, the results showed that *t* significantly impacted the relative abundance of population A (p=0.0006391) and its relaxation time, T_A (p=0.02770). The relative abundance of population A showed a linear increase of approx. 0.3% during the kneading step, from 79.39% to 79.68%; both the samples are represented as parallel straight lines with a positive and constant slope (Fig. 1a). In a similar manner to the relative abundance of population A, T_A showed a significant and linear increase during the kneading time, represented in Fig. 1b by the positive slope that characterised the trend of the parameter in the tested samples.

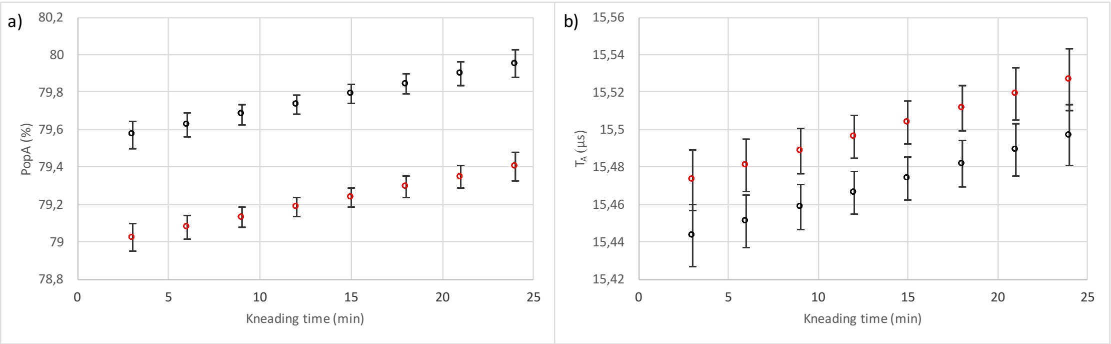

Figure 1 Kinetic models of ^{1}H NMR proton population in WWF doughs during the kneading process obtained from the experimental data of single pulse Free Induction Decay (FID): a) the relative abundance of population A, popA (%), and b) the relaxation time of population A, T_A (µs). Symbol “o” represents WWF56 (WWF dough at 56% of water content), and “o” WWF60 (WWF dough at 60% of water content). Black bars represent the 95% confidence interval of the model.

Considering the $^1HT_2$ results, a significant main effect of the kneading time was observed on the relative abundance of population C (t: p=2.878 10^{-8}), D (t: p=3.604 10^{-7}) and E (t: p<2.2 10^{-16}; t^2: p=4.066 10^{-8}) and on their relaxation times T_{2C} (t: p=0.002413), T_{2D} (t: p=4.413 10^{-8}) and T_{2E} (t: p=6.915 10^{-8}). These parameters showed a similar trend: an increase in the relative abundances and a simultaneous decrease in the relaxation times (Figs. 2a,b,c and Figs. 3a,b,c).

The relative abundance of populations C and D was significantly impacted by the first-order coefficient of t: the parameters showed a linear increase during the kneading time. The relative abundance of population C grew from 8.85% to 9.60%, whereas that of population D was affected by the interaction $t*TW$, hence it is discussed in the next paragraph. This effect can be graphically observed in the positive slope of both parameters in the tested samples throughout the process (Figs. 2a,b). With regard to T_{2C} and T_{2D}, the results showed a linear decrease in both parameters throughout the process, graphically represented by the negative constant slope that characterised the trend of these relaxation times in the tested batches of dough (Figs. 3a,b). Specifically, T_{2C} decreased from a mean value of 0.33 ms to 0.27 ms, and T_{2D} reduced from 3.67 ms to 3.43 ms.

The relative abundance of population E was affected by the first- and second-order coefficient of the kneading time (i.e., t and t^2) whereas its relaxation time T_{2E} was significantly impacted only by the first-order coefficient of t. This means that the relative abundance of population E increased as a parabolic curve, revealing a greater rise at the beginning of the process followed by a lower increase (Fig. 2c). Conversely, T_{2E} showed a linear downward trend, decreasing from 14.43 ms to 13.15 ms (Fig. 3c).

The relative abundance of population F was significantly affected by t and t^2 (p<2.2 10^{-16} and p=0.003156, respectively) and its relaxation time T_{2F} by t (p<2.2 10^{-16}). These parameters showed the opposite behaviour compared to the proton distributions C, D and E. The relative abundance of population F showed a parabolic reduction throughout the kneading step (from 11.26% to 6.77%) (Fig. 2d), whereas T_{2F} revealed an upward linear trend (increasing from 43.88 ms to 49.86 ms) (Fig. 3d).

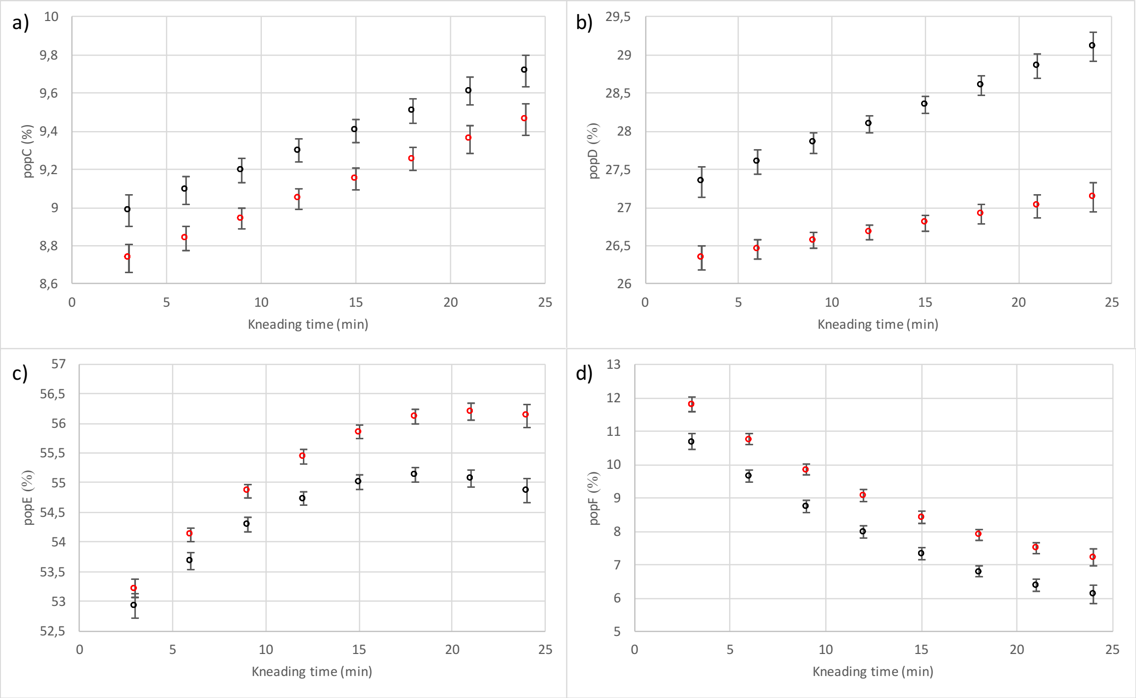

Figure 2 Kinetic models of ^{1}H NMR proton populations in WWF doughs during the kneading process obtained from the experimental data of Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence: a) the relative abundance of population C, popC (%), b) the relative abundance of population D, popD (%), c) the relative abundance of population E, popE (%), d) the relative abundance of population F, popF (%). Symbol “o” represents WWF56 (WWF dough at 56% of water content), and “o” WWF60 (WWF dough at 60% of water content). Black bars represent the 95% confidence interval of the model.

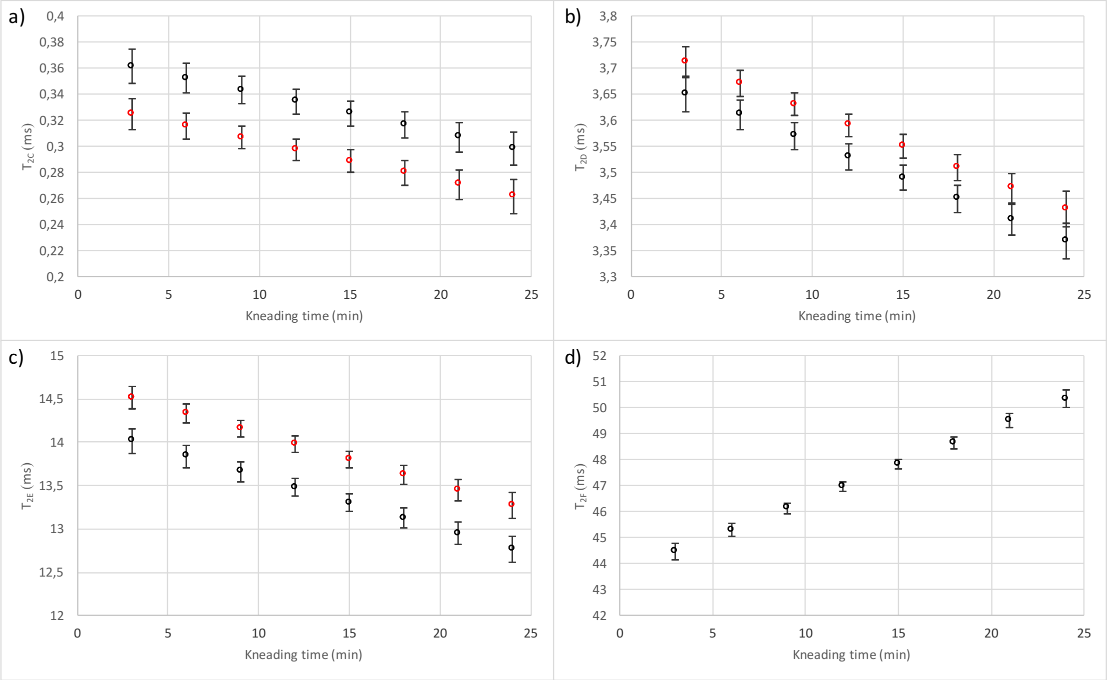

Figure 3 Kinetic models of ^{1}H NMR proton distributions in WWF doughs during the kneading process obtained from the experimental data of Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence: a) the relaxation time of population C, T_{2C} (ms), b) the relaxation time of population D, T_{2D} (ms), c) the relaxation time of population E, T_{2E} (ms), d) the relaxation time of population F, T_{2F} (ms). Symbol “o” represents WWF56 (WWF dough at 56% of water content), and “o” WWF60 (WWF dough at 60% of water content). In graph “d” the tested doughs overlapped and are both represented as “o”. Black bars represent the 95% confidence interval of the model.

3.3 The effect of total water content

The total water content of the dough, i.e., the variable *TW*, significantly affected the ^{1}H NMR distributions. The main effect of *TW* showed similar results on the relative abundance of populations A (p=9.068 10^{-12}), C (p=0.0003139) and D (p=3.487 10^{-16}): the WWF56 samples were characterised by a higher relative abundance of these proton populations than the WWF60 samples. This effect can be graphically observed by the higher value of the intercept in the batches of dough with the lower water content (WWF56) compared to the ones with the higher water content (WWF60) (Figs. 1a and 2a, b). The values of the WWF56 samples compared to the values of the WWF60 samples were 79.75% *vs* 79.25%, and 9.33% *vs* 9.09%, for populations A and C, respectively. The relative abundance of population D was also affected by the interaction *t*TW* (p=0.04557) and it showed a higher increase in the WWF56 samples than in the WWF60 batches of dough during kneading. Specifically, the values of the WWF56 samples compared to the WWF60 samples at the beginning and at the end of the kneading step were 27.36% *vs*

26.20% and 29.28% *vs* 27.12%, respectively. In Fig. 2b this effect is represented by the steeper slope of the trend of the parameter in the batches of dough with the lower water content (WWF56) compared to those with the higher water content (WWF60) during kneading.

The effect of *TW* on the relative abundances of populations E (p=0.0001011) and F (p=1.735 10^{-10}) was the opposite compared to populations A, C and D: the WWF60 samples were characterised by higher values of both parameters compared to the WWF56 samples. Indeed, the batches of dough with the higher water content (WWF60) revealed a greater intercept value compared to the samples with the lower water content (WWF56) (Figs. 2c, d). The relative abundance of population F was 8.98% in the WWF60 samples and 7.96% in the WWF56 samples. The relative abundance of population E was also affected by the interaction *TW*t* (p=0.0155801): the WWF60 samples were characterised by a higher increase in the parameter during the kneading step than the WWF56 samples. At the beginning of the process, the value of the parameter was similar for the two different water contents: the WWF56 samples showed a value of 53.02% and in the WWF60 samples the parameter was 53.22%. However, during kneading, the two samples showed a different trend in the parameter in function of the total water content: in the WWF60 samples the relative amount of population E continued increasing up to 56.08% (21 min), whereas in the WWF56 samples the parameter reached a maximum at lower values (54.78%) (at approx. 18 min). This effect is graphically represented by the steeper slope of the trend of the parameter in the WWF60 batches of dough compared to the WWF56 batches of dough (Fig. 2c)

Considering the significant effect of *TW* on the relaxation times of the proton distributions, the results highlighted that the WWF60 samples were characterised by a significant increase in T_{2E} (p=9.614 10^{-5}) compared to the WWF56 samples, whereas the opposite effect was obtained for T_{2C} (p=0.004712). These effects can be graphically observed in Fig. 1b and Fig. 3c: the trend of T_{2E} showed a greater intercept in the batches of dough with the higher water content (WWF60) than in the ones with the lower water content (WWF56), while T_{2C} showed a greater intercept in the WWF56 compared to the WWF60 batches of dough. The T_{2E} values were 13.84 ms in the WWF60 samples and 13.39 ms in the WWF56 samples. Conversely, the T_{2C} values were significantly lower in the WWF60 samples than in the WWF56 samples, at 0.29 ms and 0.33 ms, respectively.

4. Discussion

The present study applied the LR ^{1}H NMR technique to describe the proton distributions and dynamics of a WWF dough during the kneading step of breadmaking process. Scant information is given in the literature on the molecular proton changes during dough kneading (Kim, & Cornillon, 2001; Sangpring, Fukuoka, Ban, Oishi, & Sakai, 2017). Indeed, while Kim, & Cornillon (2001) investigated ^{1}H NMR molecular mobility on dough produced using three different final mixing times (i.e., 3, 18 and 30 min), Sangpring, Fukuoka, Ban, Oishi, & Sakai (2017) only monitored the first 3 min of the kneading step.

The theoretical state diagram showing the physical changes as a function of temperature and water content during breadmaking can aid the discussion and interpretation of the ^{1}H molecular signal detected in the kneading step (Cuq, Yildiz, & Kokini, 2002). The first two stages of the theoretical state diagram represent the phenomena associated with the kneading step: the full hydration of the flour constituents and the mechanical energy input produced by the mixer (Cuq, Yildiz, & Kokini, 2002). The resultant effects of the hydration and mechanical energy input include (i) swelling of the starch granules and glass transition in amorphous regions of the semi-crystalline starch structure; (ii) glass transition of the proteins and their interactions through cross-links promoted by the increase in molecular mobility (Cuq, Yildiz, & Kokini, 2002). Moreover, in a study of the rheology of wheat flour dough during the kneading step (Gras, Carpenter, & Anderssen, 2000), the evolving rheological properties of the dough in effect monitored the molecular processes in terms of bounded and unbounded water within the flour constituents occurring during mixing.

Although we studied a WWF dough system, since the main constituents of WWF are the starch and protein components, and since in the literature the presence of milling by-products in WWF dough is not seen to significantly modify the relaxation times of the proton populations (Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin, 2017b), at first we discuss the proton attributions based on the most relevant reference (Bosmans, Lagrain, Deleu, Fierens, Hills, & Delcour, 2012), then we focus on the influence of the milling by-products (Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin, 2017b).

4.1 Proton attributions and the effect of the kneading time on ^{1}H NMR kinetics

In this study, the kneading time significantly affected all the NMR parameters detected in the FID and CPMG experiments. The most rigid and abundant FID population

(population A), showed a slight but significant increase in its relative amount and mobility during kneading (Fig.1 a,b). According to the literature (Bosmans, Lagrain, Deleu, Fierens, Hills, & Delcour, 2012), this protons population is assigned to the CH protons of crystalline starch, amorphous starch and gluten not in contact with water. Since starch is the main component of the WWF dough system, it could be hypothesised that the changes in population A mainly reflected the changes in the starch structures during kneading. It is known that at room temperature and in presence of a sufficient amount of water, starch granules swell to a limited extent, adsorbing up to 46% of their dry weight of water (Goesaert, Brijs, Veraverbeke, Courtin, Gebruers, & Delcour, 2005). As a result, the volume and the surface of the starch granules grow, probably causing an increase in the starch protons exposed on the granule surface. Furthermore, the starch hydration is responsible to the glass transition of the amorphous regions which increase their molecular mobility. The significant rise in the relative amount of population A (Fig.1 a) could be interpreted as the effect of the hydration of the starch which caused the granules to swell. Instead, the significant rise in the mobility of population A (Fig.1 b) may be related to the increased mobility of the starch amorphous chains due to the glass transition as a result of kneading.

Population E was the dominant population in the CPMG proton distribution, and it showed significant changes during the kneading step (Fig. 4). Population E was assigned to the overlapped populations of starch extra-granular water and water in the gluten matrix, including mobile protons of water in exchange with hydroxyl protons of starch on the granule surface, and to water protons surrounding the sheets in exchange with gluten protons, in accordance with Bosmans, Lagrain, Deleu, Fierens, Hills, & Delcour (2012). In the literature, proton relaxing in a comparable relaxation time range of population E are the protons associated with the greatest changes during the different phases of dough processing, showing differences in function of the water content of the dough (Ruan, Wang, Chen, Fulcher, Pesheck, & Chakrabarti,1999; Doona, & Baik, 2007; Lu, & Seetharaman, 2013; Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin 2017a), storage conditions and presence of fibres (Lu, & Seetharaman, 2013), and different bread production methods (Li, Deng, Li, Liu, & Bian, 2015), as well as during the whole breadmaking process (Nivelle, Beghin, Bosmans, & Delcour, 2019). In our case too, significant changes in the relative abundance of population E were observed during the kneading time (Fig.2 c, Fig.4). At the beginning of the process, the relative abundance of population E grew at a faster rate, which could be associated with the more mobile water protons that progressively bound to the gluten proteins during the hydration phase (Fig.2 c). Indeed, in the literature data, it is reported that the dominant phase formed during hydration is represented by the water binding to the proteins (Gras, Carpenter, & Anderssen, 2000). As a consequence of the hydration of the proteins, they transited from a glassy to a rubbery state (Cuq, Yildiz, & Kokini, 2002). After the initial fast increase, the relative amount of population E showed a parabolic trend, coming close to a peak in the last stages of the process (Fig.2 c). This behaviour could be associated with the phenomena occurring after the gluten proteins have adsorbed a sufficient amount of water: the development of the gluten network through cross-linking interactions. The trend shown by the relaxation time of population E further confirmed this hypothesis (Fig. 3c). The parameter significantly decreased during kneading, revealing that the water protons of population E became progressively more tightly bound to the flour constituents, primarily represented by the gluten proteins. Furthermore, the constant decrease in the parameter after the initial hydration phase could be ascribed to the development of the gluten network, which enhances the molecular organisation, possibly explaining the shift in the relaxation time towards lower values. The NMR parameters of populations C and D changed in a linear manner during kneading: there was a significant increase in the relative amount of protons with a concurrent significant reduction in their relaxation time (Fig.2 a,b; Fig3 a,b). The increase in the relative amounts of these proton distributions was characterised by a lower growth and followed a linear trend unlike the parabolic trend of population E. According to the research by Bosmans, Lagrain, Deleu, Fierens, Hills, & Delcour (2012), these populations were assigned to some CH protons of amorphous starch and CH protons of gluten in the sheets with little contact with the confined water (population C) and to hydroxyl protons of intra-granular water and starch. In addition, they were also assigned to some CH protons of gluten and exchanging protons of confined water and gluten (population D). Hence, our results showed that, during the kneading step, the water protons bound to populations C and D increased and became more strongly bound to the flour constituents.

The most mobile CPMG population, population F, revealed marked changes in the NMR parameters during the process (Fig.2 d; Fig.3 d). Interestingly, the trend observed for this population was exactly the opposite to that of population E. Indeed, the relative amount of population F showed a decreasing parabolic trend: in the initial phase of the kneading, the parameter showed a greater decrease, whereas in the last phases it reduced to a smaller extent (Fig.2 d). In the literature, the most mobile proton population, identified as population F in the present study, has been differently attributed. Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin (2017a), who studied proton molecular dynamics and distributions in wheat flour dough and milling by-products, and Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin (2017b), who investigated ^{1}H populations in bran-enriched doughs compared to refined doughs, assigned the most mobile population to the water protons bound to the flour lipids present in the bran and germ fractions (relaxation time of approx. 100 ms). On the other hand, Assifaoui, Champion, Chiotelli, & Verel (2006) and Serial et al. (2016) attributed the most mobile population detected in biscuit dough systems to the lipids in the biscuit formula (relaxation time of 100 ms and 100-1000 ms, respectively). Conversely, Lu, & Seetharaman (2013), Li, Deng, Li, Liu, & Bian (2015) and Wang, Ye, Li, Wei, Chen, & Zhao (2017), who studied wheat doughs from refined and fibre-enriched flours, attributed the most mobile proton population to weakly bound protons of water (relaxation time of 100 ms, 30-100 ms and 37-115 ms, respectively). Our results (relaxation time of population F and its trend during the kneading step) are consistent with what is reported by Lu, & Seetharaman (2013), Li, Deng, Li, Liu, & Bian (2015) and Wang, Ye, Li, Wei, Chen, & Zhao (2017), supporting the hypothesis that this proton distribution corresponds to weakly bound protons of water. Indeed, at the beginning of the kneading, before the hydration of the flour occurred, this proton population showed the highest relative abundance as compared to final kneading times (Fig.2 d). Hence, it could be hypothesised that the protons of population F progressively bound to the flour constituents, mainly those of population E, followed by populations C and D, as shown by the significant increase in their relative amounts. Indeed, Wesley, Larsen, Osborne, & Skerritt (1998) reported that unbound water decreased quite rapidly during the hydration phase. As further confirmation, the sum of the rise in the relative amount of populations C, D and E corresponded approximately to the reduction of the relative amount of population F. The relaxation time of population F showed the opposite trend to the other CPMG populations: it increased linearly during the kneading step, revealing that these water protons became progressively more mobile (Fig.3 d). This means that weakly bound protons of water become progressively not only less abundant but also more mobile during kneading. This weakly bound fraction of water that is retained at the end of the kneading phase could have an important role in the plasticization of the dough structure. A schematic representation of the authors' interpretation of ^{1}H NMR results as a function of the kneading time is reported in Fig. 5.

Our WWF dough system contained both the bran and the germ fractions, which contributed to the observed ^{1}H NMR signals. The literature contains scant information on the proton molecular dynamics and mobility in a similar dough system (Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin, 2017b). Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin (2017b) have studied the influence of the bran fractions on water mobility and biopolymer behaviour during breadmaking and storage. Their results showed that FID and CPMG proton populations in the bran-enriched dough after the mixing step relaxed in the same relaxation times range as populations found in the refined dough sample used as a control (Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin, 2017b). The main observed differences were the relative abundance of the proton populations: popA was approx. 16-20% less abundant in the bran-enriched dough, and this effect was related to the greater amount of water required by these doughs compared to the control sample (Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin, 2017b). The CPMG signal revealed a higher relative amount of popC, E and F in the bran-enriched doughs and a greater relative abundance of popD in the control sample (Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin, 2017b). The different proton distributions in the bran-enriched dough can be assigned to CH protons of amorphous starch and dietary fibre constituents such as arabinoxylan (popC), exchanging protons of bran- and flour- related biopolymers and water interacting with these biopolymers outside the starch granules (popE), and protons originating from lipids (popF) (Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin, 2017b). The higher popD observed in the refined sample was assigned to CH protons of gluten and exchanging protons of gluten and of starch, and water inside the starch granules (Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin, 2017b). Hence, in our WWF dough samples the presence of milling by-products, although in a smaller amount compared to the main flour constituents, could have contributed to the characteristic ^{1}H NMR profile. Furthermore, since the bran showed a high water-binding capacity (Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin, 2017a,b), it could have impacted the results obtained as a function of the total water content.

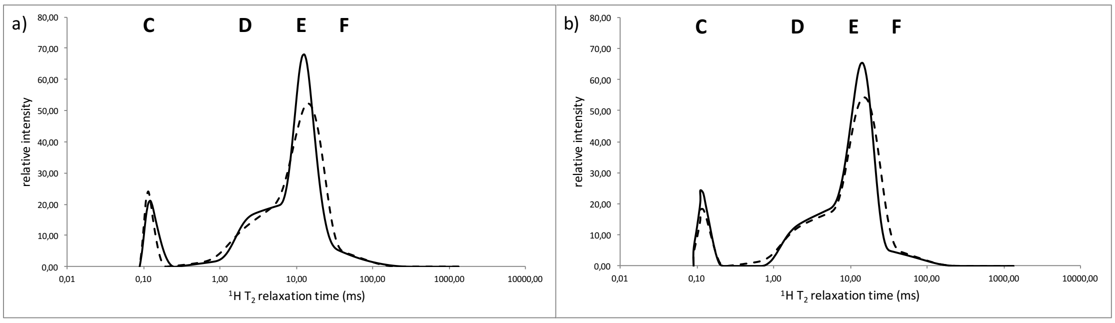

Figure 4 Representative CPMG proton molecular distributions of the WWF dough samples during the kneading step: a) WWF dough samples containing 56% of water content (the WWF56 samples), b) WWF dough samples containing 60% of water content (the WWF60 samples). Dashed lines represent the dough samples after 6 min of the kneading step and solid lines the dough samples at the kneading time where the highest amount the dominant population (popE) was reached (t=18 min in the WWF56 samples, t=21 min in the WWF60 samples).

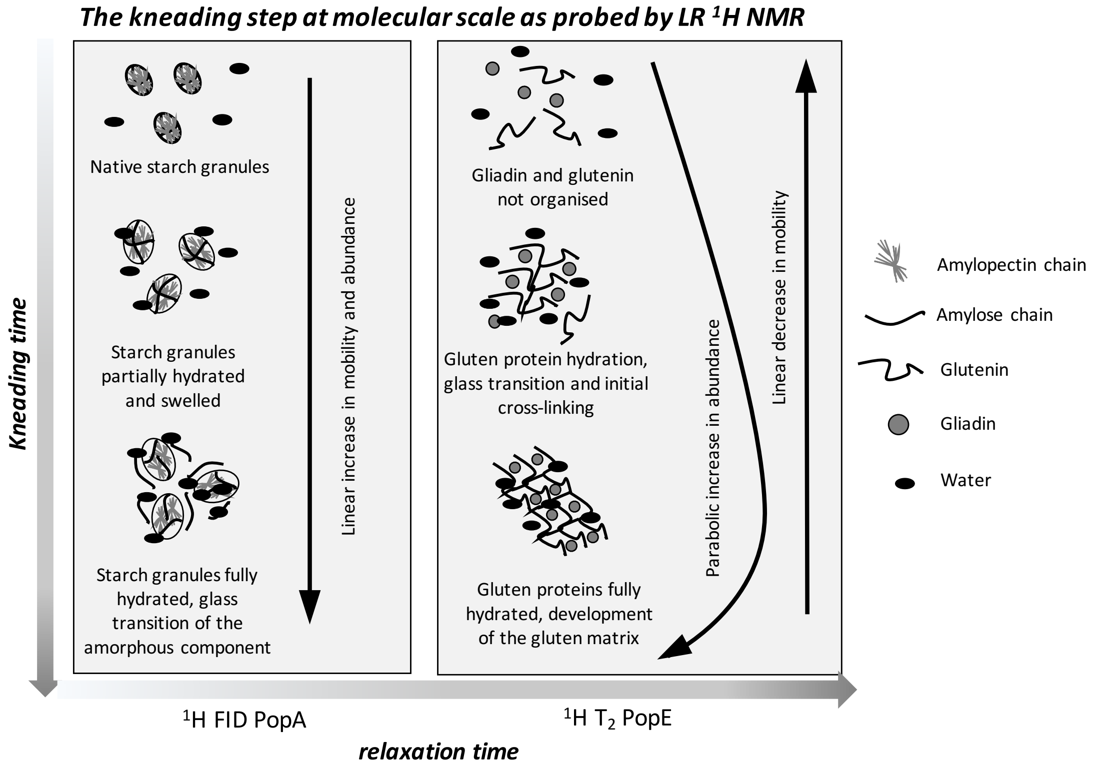

Figure 5 Schematic representation of the authors' interpretation of the ^1H NMR results obtained during the dough kneading step.

4.2 *The effect of total water content on ^1H NMR kinetics*

The total water content of the dough significantly affected the NMR parameters. The two levels of water tested were 56% and 60% (w/flour w) which corresponded to a total dough hydration of 43.65% and 45.06%, respectively. The batches of dough with a 56% water content were characterised by a higher relative amount of populations A, C and D (Fig.1 a; Fig.2 a,b), a higher relaxation time of population C (Fig.3 a), and a greater increase in the mobility of population A (Fig.1 b) and in the relative amount of population D during kneading (Fig.3 b). Conversely, the batches of dough with a 60% water content showed a higher relative abundance of populations E and F (Fig.2 c,d), a greater relaxation time of the prevalent populations A and E (Fig.1 b; Fig.3 c), and a higher increase in population E during kneading (Fig.2 c).

These results revealed that a change in the total dough water content of approx. 1.4% produced significant differences among the proton distributions within the dough system. Several studies have investigated the effect of different dough water contents on ^{1}H NMR molecular dynamics and mobility (Ruan, Wang, Chen, Fulcher, Pesheck, & Chakrabarti,1999; Choi, & Kerr, 2003; Wang, Choi, & Kerr, 2004; Assifaoui, Champion, Chiotelli, & Verel, 2006; Doona, & Baik, 2007; Lu, & Seetharaman, 2013). However, to the best of the authors' knowledge no studies have tested the effect of the dough water content during the kneading step.

Similar results showing a decrease in the relative amount of the less mobile detected population (approximately our popA) alongside an increase in the dough water content have been reported in the literature and associated with the complete hydration of all the water-binding sites on the flour solids (Ruan, Wang, Chen, Fulcher, Pesheck, & Chakrabarti,1999; Choi, & Kerr, 2003; Doona, & Baik, 2007; Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin, 2017b).

The effects observed in the present study on CPMG proton distributions indicated that the total water content produced significant differences in the redistribution pattern of the water molecules among the flour constituents. The higher the water availability (60% batches of dough), the more the protons belonging to the more mobile proton populations E and F and the higher the mobility of population E. Furthermore, the mobility of population C decreased. Figure 2c clearly shows that the relative amount of population E peaked at different kneading times in function of the total water content: the less hydrated dough (56% batches of dough) reached the maximum value of the parameter at approx. minute 18 of the kneading step. Conversely, in the more hydrated sample (60% batches of dough), the relative abundance of population E continued to increase throughout the process, reaching maximum level at minute 21 of the process, at a higher value than what was observed in the less hydrated dough (Figure 2c). This result can be also observed in Figure 4, showing the representative proton T_2 distributions of the WWF dough samples obtained at two different kneading times: (i) after 6 minutes of the kneading step for both 56% and 60% water doughs and (ii) at the kneading time corresponding to the highest relative amount of popE (i.e. 18 min in 56% water doughs, 20 min in 60% water doughs). In the literature, it is known that the farinographic test cannot correctly predict the water absorption of WWF, and usually a higher water content is required to improve product quality (Bruckner, Habernicht, Carlson, Wichman, & Talbert, 2001; Hemdane, Jacobs, Dornez, Verspreet, Delcour, & Courtin, 2016). Indeed, it has been found that the presence of fibres not only causes a dilution effect on the gluten proteins, but fibres also have a strong water binding capacity that negatively affects dough development owing to several phenomena which are not yet fully understood (Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin, 2017b).

Our results disclosed that in WWF dough systems a higher water content produces a greater hydration of the biopolymers belonging to population E. This data may highlight that a higher water availability produced a better hydration of the gluten proteins by reducing their competition for water molecules with the bran fractions relaxing in this relaxation time range (Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin, 2017b). Furthermore, a higher amount of weakly bound water (population F) was observed in WWF doughs at the higher water content. Both these effects may account for the positive role of greater hydration on WWF dough, which is generally associated with an improvement in the flour's technological properties and bread quality (Bruckner, Habernicht, Carlson, Wichman, & Talbert, 2001; Hemdane, Jacobs, Dornez, Verspreet, Delcour, & Courtin, 2016). Furthermore, it can also be speculated that the time required for the WWF system to reach full hydration is longer when a higher amount of water is available, as suggested by the different maximum values shown by the dough samples (18 min and 21 min, respectively).

5. Conclusions

In this study, ^{1}H NMR was applied for the first time to monitor the proton mobility and dynamics in wholewheat flour based dough, as a function of the kneading time and total water content.

A significant effect of the kneading time was observed on all ^{1}H NMR parameters and results were interpreted as physical/chemical phenomena occurring during kneading. The protons belonging to the less mobile population (population A) and to the two most mobile populations, (population E and F) showed the major changes, interpreted in terms of starch granules swelling, glass transition of amorphous starch regions, proteins hydration and gluten formation, and gradual decrease of free water during kneading.

The effect of total water content may have revealed the molecular insights making the use of high water amounts a key factor for WWF based products. The increase in the total water content led to a significant increase of the relative abundance of the most mobile populations (i.e., E and F), and to a growth in the mobility of populations A and E. The higher hydration of gluten proteins and higher free water fraction may be responsible for the general improvement of WWF dough and bread properties when higher water amounts than that predicted by the Farinograph are used. However, other experiments are required to confirm these hypotheses. In this light, the use of ^{1}H NMR may help to better understand the molecular dynamics within the dough system so as to discover innovative processing strategies specifically adapted to the different characteristics of WWF.

This first study of the use of ^{1}H NMR to monitor the kneading step of breadmaking has shown that this technique is able to detect the main chemical/physical phenomena occurring during kneading. This encourages its further application in order to correlate the molecular pattern of dough to the physical characteristics of the final product.

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5. Conclusions and Future developments

The introduction of the roller mill made it possible to separate the three main components of the wheat kernels (i.e. the endosperm, bran and germ) at the beginning of the process, allowing to obtain highly refined flours from the starchy endosperm and to standardize the bread-making process (Jones et al., 2015; Zhou et al., 2014; Cauvain et al., 2015; Parenti et al., 2020a). At present time, there has been a renewed interest for unrefined flours, due to the positive effects that they have shown on human health (Hauner et al., 2012; Ye et al., 2012). The presence of milling by-products (i.e., bran and germ) significantly changes the flour chemical composition, improving its nutritional quality, whereas showing detrimental effects on the technological performance (Hemdane et al., 2016; Boukid et al., 2018). Although there has been an increasing interest for the use of unrefined flours in bread-making, in the literature the standard tests to determine technological quality have remained unvaried. On the other hand, the main strategies the scientific literature has proposed to improve the bread-making performance of unrefined flours are based on the use of improvers, followed by the use of the sourdough fermentation and pre-treatments of the milling by-products. Few studies have investigated modifications of the processing conditions as a function of the different requirements of unrefined flours compared to refined flours (Parenti et al., 2020a).

After the above background, a survey article was performed with the aim to obtain an empirical state of the art about the main strategies adopted by bakers for the bread-making process with unrefined wheat flours. We decided to focus on “old wheat flours”, those varieties cultivated before the intensive technological selection occurred in the late 1960s of the Green Revolution, since an increasing interest for this flour category, as a result of the scientific studies showing the health benefits of some of these cultivars (Leoncini et al., 2012; Dinelli et al., 2011; Sofi et al., 2010; Gotti et al., 2018; Sereni et al., 2017), has been observed on the market. Results showed that old wheat flours are processed following “good working practices” during the whole production chain, starting from the field to the bread-making process. As a consequence, the use of these flours results in positive effects on the environment as well as on human health. Bakers are not used to add additional ingredients in the bread formula, since consumers demand for clean-label products. Conversely, they have developed processing strategies to improve the technological performance of unrefined bread, including the use of short mixing times, the identification of the correct leavening time, the inclusion of flour blends in the dough formula, the use of high water amounts, the maintenance of the sourdough viability. All bakers required the introduction of new processing techniques able to improve the breadmaking performance of unrefined flours while preserving their nutritional value (Guerrini et al., 2019).

The following study focused on unrefined bread formula: the effect of changing the physical form in a part of the recipe flour was investigated on dough properties and bread quality. The pre-treatment consisted of performing a pre-gelatinisation of part of the total dough flour the day before the bread-making test. Pre-gelatinized flour strongly interacted with water, increasing the optimum water amount to make dough. Positive results were obtained on dough rheological properties in terms of dough strength and tenacity to extensibility ratio. Bread quality was significantly improved, showing higher volume and better texture parameters, preserved during 48 h of storage. The inclusion of pre-gelatinised flour could represent an interesting technique to obtain clean-label bread from unrefined flours (Parenti et al., 2019).

After this study, considering the variable chemical composition of unrefined flours, we decided to use a different approach for optimizing the bread formula. We realized that the effects of common bread improvers were investigated on refined flours as well as the simultaneous effect of different improvers has not been tested before. Therefore, we decided to test the effect of natural bread improvers on the bread-making performance of an unrefined wheat flour. A two-step experiment (i.e. a screening design followed by a validation trial) in order to identify the combination of improvers showing the best effects on dough properties and bread quality was performed. The most positive effects on the technological performance of the specific whole-wheat flour tested were observed with the use of sucrose, ice and EVOO. Sucrose and EVOO were able alone to improve dough rheological properties, whereas the combined inclusion of both the improvers was required to improve the overall bread quality (Parenti et al., 2020b).

After the focus on the bread formula, we decided to expand our research on the improvement of unrefined wheat flour performance focusing on the processing variables. Kneading is the first step of the process, considered as one of the most important, during which several physical-chemical transformations occur to the flour constituents (Cuq et al., 2003; Zhou et al., 2014; Cauvain et al., 2015; Guerrini et al., 2019). Recently ^{1}H NMR technique has been successfully applied in the food industry, showing interesting results during the processing of cereal based products (Curti et al., 2013; Curti et al., 2015; Bosmans et al., 2016; Hatzakis, 2019). However, this technique has never been applied to gain a molecular insight of the physical-chemical phenomena occurring during dough kneading. Therefore, we decided to use ^{1}H NMR technique to describe the dough development of a whole-wheat dough as a function of water amount. The major changes observed as a function of time were interpreted as starch granule swelling, glass transition of amorphous starch regions, protein hydration and gluten formation, and gradual decrease of free water during kneading. As water content increased, a higher hydration of the gluten proteins (population E) and the higher "free" water (population F) were observed which may be responsible for the general improvement of unrefined dough and bread quality when higher water amounts than that determined with Farinograph are used. The use of ^{1}H NMR may help to better understand the molecular dynamics within the dough system with the aim to find innovative processing strategies adapted to the inherent characteristics of unrefined flours. This study showed that ^{1}H NMR technique is able to reveal the main physical-chemical phenomena during kneading, hence encouraging to find correlations between molecular pattern of the dough and physical characteristics of dough and bread (Parenti et al., 2021).

Future developments of the thesis research involved the investigation of a widespread practice performed by bakers during the kneading step: the gradual addition of basic bread ingredients (i.e. flour and water). Since in the literature few studies have tested the effect of changing the order of ingredients addition during kneading, we decided to investigate this practice on dough molecular properties (using ^{1}H NMR technique), dough rheology and bread quality (Parenti et al., submitted to *Journal of Food Engineering*).

Furthermore, the focus on the kneading step continued considering the importance that dough development status has on product quality: in the literature it is widely reported that under- and over- mixed doughs show poor properties and result in low-quality breads. With regard to unrefined doughs, the poor stability at kneading and short development time usually observed make the proper evaluation of the dough development even more important for bread quality. Hence, we performed a literature research about all the available methods to determine the optimum dough development. Both standard and alternative methods were reviewed, and their weaknesses and strengths were outlined. Finally, the most promising methods were identified as a function of the application field, the scientific research and baking industry (Parenti et al., submitted to *Journal of Food Engineering*)

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