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Optimization of the malting process of Calabrian cereal landraces through response surface methodology

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Abstract

The final purpose of the thesis work outlined below was to optimize the malting process of two local populations of wheat and rye, also known as landraces, grown in the Calabrian territory (south Italy). The research carried out consisted of three parts: a two-year agronomic study; a preliminary assessment of the malting aptitude of the chosen cereal species; and the optimization of the malting process of two landraces of wheat and rye using the surface response methodology. Thus, the first part consisted of an agronomic study aimed at characterizing the bio-agronomic traits of malting barley varieties and local populations of wheat and rye to verify their agronomic potential, as well as their production stability in the test environment. A randomized block experimental design with four replications was chosen for conducting the field experiments and subsequent data analysis. Hence, three different cereal species were compared, with the following purposes: in the case of malting barley, the aim was to evaluate over the two years the productive and qualitative potential of the chosen varieties in the field, whose agronomic performances are well established; in the case of wheat and rye, in addition to what has already been stated above, the aim was to understand which of the landraces would be better suited to the next phase of the study. Briefly, after two years of cultivation in the same test area, the effect of the environment and its interaction with the varieties was seen to be significant: in particular, a general decrease in yield and the other qualitative and purely agronomic traits was estimated. In fact, the delay of the sowing time in the second growing season, due to adverse meteorological conditions, negatively affected the earing phase for example, with consequent adverse effects on the further responses examined. At the same time, although the local varieties were similarly affected, the intensity of the decrease in the analyzed variables was smaller in their case, thus leading to the conclusion that the local populations, which are well adapted to the test environment, showed greater resilience to external sources of variability. Once harvesting was completed in the first year, the grain of each variety was subjected to the next step of the study for an initial screening of their malting suitability. Therefore, during the second year of the doctoral project, malting tests and consecutive qualitative malt analyses were carried in collaboration with the Italian Brewing Research Centre (CERB), University of Perugia, Italy. All varieties from the experimental field were subjected to the same malting schedule. In particular, rye was subjected to a special processing, having undergone different days of germination. Compared to the information in the literature on the first two cereals, a certain lack distinguishes rye, so this choice was justified by the desire to observe, prior to the process optimization experiments, how one of the characteristic malting parameters could affect the transformation of the raw material under investigation. The two-row barley varieties presented excellent characteristics on average as expected, in full agreement with the quality standards for malting barley. Different situation occurred for wheat and rye landraces, which were subjected to such a biotechnological process for the first time, within the limits of our knowledge, presenting inferior characteristics. This did not arouse any concern, as these local populations have traditionally been used for breadmaking in Calabria and have not generally received any genetic improvement to enhance their performance for the processing industry, whether breadmaking or malting. Nevertheless, these preliminary tests served as the basis for choosing two varieties of wheat and rye from those under study in order to further subject them to a malting optimization process. The third phase of the project was carried out in Germany at the Weihenstephan Research Center for Brewing and Food Quality, Technical University of Munich. During the doctoral year abroad, precisely, specific malting trials were conducted with the aim of increasing the malting performance of the two landraces. For this purpose, the response surface methodology (RSM) was used to create and analyze experimental designs to evaluate the effect of three malting parameters on the compositional characteristics of the chosen grains. Germination time, germination temperature and steeping degree were chosen as process parameters. They were set at three levels using a face-centered composite design. This methodology, which is widely used in the field of biotechnology, made it possible to evaluate and predict how the responses of interest, with respect to proteolytic, cytolytic and amylolytic

modifications of the derived malt samples, varied according to different combinations of the parameters through response surface analysis. The actual optimization analysis was carried out by means of a desirability function in the case of wheat and according to the same approach combined with a genetic algorithm in the case of rye. In conclusion, the implemented methodology allowed for the improvement of the malting process of the two landraces in relation to specific quality traits.

Riassunto

Il lavoro di tesi di seguito illustrato ha avuto come obiettivo finale l'ottimizzazione del processo di maltazione di due popolazioni locali, note anche come landraces, di frumento e segale coltivate nel territorio calabrese (sud Italia). La ricerca effettuata è stata composta da tre parti: uno studio agronomico biennale; una valutazione preliminare dell'attitudine alla maltazione delle specie di cereali scelte; l'ottimizzazione del processo di maltazione di due landraces di frumento e segale per mezzo della metodologia di risposta di superficie. La prima parte è, quindi, stata costituita da uno studio agronomico finalizzato alla caratterizzazione dei tratti bio-agronomici di varietà di orzo da malto e popolazioni locali di frumento e segale per verificarne la potenzialità agronomica, insieme alla stabilità produttiva nell'ambiente di prova. Un disegno sperimentale a blocchi randomizzati con quattro repliche è stato scelto per la conduzione delle prove di campo e la consecutiva analisi dei dati. Per cui, tre diverse specie di cereali sono stati confrontati, con le seguenti finalità: nel caso dell'orzo da malto, l'intenzione è stata quella di valutare, nel corso dei due anni, le capacità produttive e qualitative delle varietà scelte in campo, le cui performance agronomiche sono ben consolidate; nel caso del frumento e della segale, oltre a quanto già precedentemente esposto, comprendere quali delle landraces avrebbe potuto meglio prestarsi alla fase successiva dello studio. Sommarariamente, dopo due anni di coltivazione nella medesima area di prova, si è visto che l'effetto dell'ambiente e della sua interazione con le varietà è risultato significativo: in particolare, un generale decremento in termini di resa e degli altri caratteri qualitativi e prettamente agronomici valutati è stato stimato. Il ritardo della fase di semina, causa condizioni climatiche avverse, ha infatti negativamente influito sulla fase di spigatura ad esempio, che è risultata in ritardo rispetto al primo anno, con conseguenti effetti avversi sulle ulteriori risposte esaminate. Al contempo, sebbene anche le varietà locali abbiano subito la medesima influenza, l'intensità del decremento delle variabili analizzate è risultato più contenuto nel loro caso, potendo così concludere che le popolazioni locali, ben adattate all'ambiente di prova, abbiano mostrato una maggior resilienza nei confronti delle fonti di variabilità esterna. Una volta effettuata la raccolta al primo anno, la granella di ciascuna varietà è stata sottoposta a maltazione per un primo screening della loro attitudine maltaria. Per cui, durante il secondo anno di dottorato, delle prove di maltazione e delle consecutive analisi qualitative sul malto sono state effettuate in collaborazione con il Centro di Ricerca per l'eccellenza della Birra (CERB), Università degli Studi di Perugia (Italia). Tutte le varietà provenienti dal campo sperimentale sono state sottoposte al medesimo processo. In particolare, la segale ha subito un trattamento particolare, essendo stata sottoposta a diversi giorni di germinazione. Rispetto alle informazioni presenti in letteratura sui primi due cereali, una certa carenza caratterizza la segale, per cui tale scelta è stata giustificata dalla volontà di osservare preliminarmente alle prove di ottimizzazione di processo, come uno dei caratteristici parametri di maltazione possa influire sulla trasformazione della materia prima in esame. Gli orzi distici, come previsto, hanno presentato caratteristiche in media eccellenti, in pieno accordo con gli standard qualitativi per il cereale. Situazione differente si è verificata per le landraces di frumento e segale, per la prima volta sottoposte a tale processo biotecnologico entro i limiti della nostra conoscenza, che hanno presentato caratteristiche inferiori. Ciò non ha destato alcuna novità, essendo questa popolazioni locali tradizionalmente utilizzate in panificazione in Calabria e non avendo subito in generale alcun miglioramento genetico atto a potenziarne le performance per l'industria della trasformazione, sia essa quella della panificazione o della maltazione ancor di più. Nonostante ciò, tali prove preliminari sono servite come base per scegliere due varietà di frumento e segale tra quelle allo studio, in modo da sottoporle ad un processo di ottimizzazione. La terza fase del progetto è stata effettuata in Germania presso il Research Center Weihenstephan for Brewing and Food Quality. Durante l'anno di dottorato all'estero, appunto, prove di maltazione specifiche sono state condotte con lo scopo di incrementare le performance maltarie di due landraces. A tale scopo, la metodologia di risposta di superficie è stata utilizzata per la creazione e l'analisi di disegni sperimentali che hanno permesso di valutare l'effetto di tre

variabili di processo durante la maltazione sulle caratteristiche compositive dei cereali prescelti. Il tempo di germinazione, la temperatura di germinazione e l'umidità del cereale sono stati scelti come parametri di processo. Essi sono stati impostati su tre livelli utilizzando un disegno composito a facce centrate. Tale metodologia, ampiamente utilizzata nel campo delle biotecnologie, ha permesso di valutare e di predire come le risposte di interesse, relativamente alle modifiche proteolitiche, citolitiche e amilolitiche dei campioni di malto ottenuti, siano variate a seconda di diverse combinazioni dei parametri attraverso l'analisi delle superfici di risposta. L'analisi di ottimizzazione vera e propria è stata effettuata per mezzo di una funzione di desiderabilità nel caso del frumento e secondo lo stesso approccio, combinato ad un algoritmo genetico, nel caso della segale. In conclusione, la metodologia implementata ha permesso di migliorare il processo di maltazione delle due landraces in relazione a specifici tratti qualitativi.

Keywords

Barley; wheat; rye; cereal landrace; agronomic assessment; malting; response surface methodology.

Capitolo 1

General introduction

1. Local cereals for beer production

The beer market is a highly competitive sector, particularly in the case of the craft movement, which is forced to compete with industry giants and the large-scale retail trade. The Italian beer sector has undergone a peculiar evolution, traceable after the Second World War with the predominance of large companies until the 1980s, when the appearance of smaller craft breweries made their way in. One of the drivers of this gradual change was consumer demand, a key factor in the dynamics of industrial evolution (Garavaglia, 2022). In a land where wine consumption dominates and where beer appears to be secondary, even though it is part of the history dating back to the rule of the Austrian empire, consumer preference appears to be an essential factor in leading market change (Fastigi et al., 2016). Various epithets have been associated with craft firms: referring to size (small), or to the ownership of the company itself (independent or not). Another term that is commonly used is 'traditional', to indicate those breweries that make the use of local or unusual raw materials their main mission (Garavaglia & Swinnen, 2018). This trend towards differentiation is sometimes realized by the introduction of uncommon ingredients that are part of a nation's culture and tradition, e.g. in the case of the Italian style of beer IGA (Italian grape ale), that involves the addition of grapes or grape must to a common beer wort (Garavaglia, 2020). Since grains and the malts derived from them are a constituent part of beer, considerable research is conducted on new varieties or alternative grains and pseudocereals. In the Mediterranean area, a variety of cereals can be found, from modern and old varieties to local populations with a purely local distribution. The utilization of local raw materials is one of the most popular strategies for the regionalization of beer making (De Simone et al., 2021). Traditionally and long-standing cereals include the so-called landraces. Several terms have been used as synonyms for the latter term, particularly in the first half of the 1900s. Interest in the maintenance of landraces as a genetic resource was discussed on several occasions and conferences during the 20th century, to the extent that, at an international congress organized by the FAO in 1927, a recommendation was given to the participants attending, which was to maintain old populations by promoting their cultivation in their homelands. An initial definition of the term sees it in contrast to that of cultivar, from which a landrace differs by not having uniform and stable traits and not having been selected for particular characteristics to be maintained over time. Among the earliest descriptions dating back to the beginning of the 1900s, mention must be made of von Rümker's definition that a cereal landrace is one that bears the name of the area in which it has long been cultivated. Thereafter, the focus shifted to the ability of these old populations to grow even under adverse conditions as their selection, rather than by man, was carried out by nature itself. As the seasons progressed and local farmers maintained the same genotypes, these grains developed resistance traits peculiar to the area, such as tolerance to frost and drought, as well as the ability to withstand adverse temperatures (Zeven, 1998).

2. Malting

Malting is the process that consists of steeping, germinating and drying grain to convert it into malt. The etymology of the word malt derives from the Anglo-Saxon language and probably refers both to the softening of the cereal following the absorption of water and therefore to its structural modification, and to the action of grinding the cereal before its use to facilitate the solubilization of the substances contained therein. The production of malt and beer dates back to

millennia ago, so much so that there is historical evidence on the production and sale of beer in ancient civilizations such as Egypt, Rome and the Nordic countries of Europe. In some German cities, for example, malt production was regulated to such an extent that only barley and oats could undergo this process. Malt contains various substances, but certainly the potential to yield a high extract is a trait of primary importance. In fact, once properly ground and mixed with hot water according to a specific program of times and temperatures beneficial to enzymatic degradation activity, it is possible to obtain a liquid with a higher density as a result of the dissolution of malt-derived substances. Originally, malting was a small-scale and partly clandestine activity given the mandatory tax liability, performed without any technology. In North America it was customary to malt corn, although with poor results in terms of quality, so that beer brewed using bread was more popular. Another common practice for malting large quantities of cereal was floor-malting in the 17th century. If in 1800 malting was an activity carried out over a precise period of time (from November to April) with limited production capacity, with the advent of industrialization, the consequent birth of large industries and the advent of new technologies, the quantities of malt destined for large breweries have increased considerably and productions have become constant throughout the year. Malting traditionally consists of three consecutive steps: steeping, germination and kilning. In reality, other preliminary and conclusive steps are necessary to conduct the process in the most effective manner, such as cleaning and sieving the raw material and storing the dried malt in optimal conditions. Therefore, after thorough cleaning and after verifying that the dormancy of the grain is reduced to sufficient levels, the grain can be proceeded to actual malting. The first canonical stage involves alternating breaks in the water and air so that the grain can absorb it internally and at the same time avoid the occurrence of asphyxiation phenomena. The goal of this process is to raise the humidity of the cereal to levels generally between 42 and 48% under controlled temperature in order to trigger the germination phase. Commonly, the grain mass is soaked in water for set times, although the practice of spraying water can also be adopted. Since this phase also coincides with the further cleaning of the cereal, the water used must be replenished when it is excessively dirty by material extraneous to the cereal. Therefore, the absorption of water allows to soften the cereal and activate the biochemical and physiological activities that will lead each seed to growth in the next phase, although this process is already visible. Rotation of the mass during germination is important to prevent it from clumping as a result of rootlet growth and thus avert damage to the germinating seeds. During this second step, the temperature is maintained at the desired level and air is circulated to remove carbon dioxide originating from grain metabolism. It is during germination that the grain undergoes an internal modification that coincides mainly with the formation and accumulation of newly formed and partly pre-existing hydrolytic enzymes, cell wall modification resulting in softening of the caryopses. This phenomenon is externally evident with the gradual development of rootlets and acrospire. Once the target degree of modification has been reached after several days of germination, the cereal is subjected to the final phase of malting. Kilning is necessary in order to lower the moisture content of the grain, thus interrupting germination and obtaining a malt with enhanced friability. Each type of malt, from the lightest to the darkest, requires that the drying be carried out at different temperatures, in such a way that the desired color and aroma are formed and, in particular, that the enzymatic pool generated in the previous step is almost kept intact. This is not the case in dark malts, in which, due to the higher temperatures involved, the enzymatic activity is variously lessened as a result of enzyme inactivation (Briggs, 1998). A variety of aromas are formed by means of non-enzymatic browning reactions during kilning. In flavor formation, not only the effect of temperature should be considered, but also the interaction with the moisture content of the germinated grain (Prado et al., 2021). Even in the case of malting, as in a wide diversity of areas where energy and water consumption exert a certain environmental impact, research is attempting to identify environmentally friendly solutions that, among other aspects, shorten malt production times, although with related changes to the malting and brewing process. One of the proposed solutions, for example, concerns the use of undried malt, also known as green malt, for whose

production the last malting step is completely bypassed. Although this allows to reduce energy consumption, the use of undried malt involves some technical challenges, such as the presence of rootlets which negatively affect the mashing phase, subsequently altering the quality of the beer (Dugulin et al., 2020). At the end of the heat treatment, the rootlets are removed and the malt is stored in ideal conditions until use.

3. Response surface methodology

To successfully apply the scientific method, it is necessary to evaluate and adopt an appropriate experimental design. Defining the objective of the scientific investigation and organizing the necessary experiments in advance are essential stages in order to reduce errors and avoid the collection of fragmentary answers. There are different types of experimental designs, each of which is suited to a specific purpose and has the basic prerequisite of minimizing the effect of the experimental error. The most appropriate design will then guide the handling and collection of data, as well as the choice of the most suitable mathematical model for analyzing and drawing conclusions (Lawson, 2015). Setting up and conducting the micro-malting experiments were accomplished using the response surface methodology (RSM), that is defined as a “collection of mathematical and statistical techniques that are used for the development, the improvement and the optimization of processes”. This technique is commonly adopted for the improvement of existing products or the development of new ones. Its implementation normally involves the optimization of several attributes, defined as responses indeed, whose values are influenced by several factors (Myers, 2009). The methodology is so called because the variation of a response for each combination of process variables is represented by means of contour (two-dimensional) or surface (three-dimensional) plots. There are two types of models typically adopted in a response surface analysis, where a functional relationship between a response of interest and a number of variables exists: first-order and second-order models. Although this relationship is considered unknown, it can be approached with a low-order polynomial. This model provides an approximation of a relationship between variables in order to predict the change of a response as the independent variables vary within the limits of the experimental region. The consecutive objective is to determine which of the investigated factors exerts a significant effect or not, to determine the optimal conditions that allow the research objective to be met, which may be to maximize, minimize or maintain a response of interest within a certain range (Khuri & Mukhopadhyay, 2010). The execution of a surface analysis is not reduced to a simple linear regression. First of all, once the process variables and their ranges have been defined, their coding is required. Then, it is essential to assess the adequacy of the adopted mathematical model and thus correctly visualize the response surfaces (Lenth, 2009). The final step in this approach is usually to find the desired optimum by combining the different models first developed and setting the optimization problem according to the desired objective. The greater the number of responses to be combined, the more complex the solution of the optimization problem will be. Several approaches have been developed over the years, including desirability functions and genetic algorithms.

References

- Garavaglia, C. (2022). Industry evolution: Evidence from the Italian brewing industry. *Competition & Change*, 26(1), 75–95. <https://doi.org/10.1177/10245294211007408>
- Fastigi, M., Esposti, R., & Viganò, E. (2016). The irresistible rise of craft breweries in Italy: The case of agricultural craft breweries. Conference: Second International Conference on Agriculture in an Urbanizing Society At: Rome Volume: Proceedings of the conference (ISBN 978-88-908960-3-3)

Garavaglia, C., & Swinnen, J. (2018). Economic Perspectives on Craft Beer: A Revolution in the Global Beer Industry. In *Economic Perspectives on Craft Beer: A Revolution in the Global Beer Industry*. <https://doi.org/10.1007/978-3-319-58235-1>

Garavaglia, C. (2020). The Emergence of Italian Craft Breweries and the Development of Their Local Identity. In N. Hoalst-Pullen & M. W. Patterson (A c. Di), *The Geography of Beer* (pagg. 135–147). Springer International Publishing. https://doi.org/10.1007/978-3-030-41654-6_11

De Simone, N., Russo, P., Tufariello, M., Fragasso, M., Solimando, M., Capozzi, V., Grieco, F., & Spano, G. (2021). Autochthonous Biological Resources for the Production of Regional Craft Beers: Exploring Possible Contributions of Cereals, Hops, Microbes, and Other Ingredients. *Foods*, 10(8), 1831. <https://doi.org/10.3390/foods10081831>

Zeven, A. C. (1998). Landraces: A review of definitions and classifications. *Euphytica*, 104(2), 127–139. <https://doi.org/10.1023/A:1018683119237>

Briggs, D. E. *Malts and malting*, 1st ed.; Blackie Academic & Professional, an imprint of Thomson Science, 2-6 Boundary Row, London SE18HN, UK, 1998.

Prado, R., Gastl, M., & Becker, T. (2021). Aroma and color development during the production of specialty malts: A review. *Comprehensive Reviews in Food Science and Food Safety*, 20(5), 4816–4840. <https://doi.org/10.1111/1541-4337.12806>

Dugulin, C. A., Clegg, S. C., De Rouck, G., & Cook, D. J. (2020). Overcoming technical barriers to brewing with green (non-kilned) malt: A feasibility study: Overcoming technical barriers to brewing with green (non-kilned) malt: a feasibility study. *Journal of the Institute of Brewing*, 126(1), 24–34. <https://doi.org/10.1002/jib.602>

Lawson, J. (2015). *Design and Analysis of Experiments with R*. Chapter 10. Taylor & Francis Group.

Myers R. H., Montgomery D. C., Anderson-Cook C.M (2009). *Response Surface Methodology: process and product optimization using designed experiments*, 3rd ed. Wiley Series in Probability and Statistics.

Khuri, A. I., & Mukhopadhyay, S. (2010). Response surface methodology. *Wiley Interdisciplinary Reviews: Computational Statistics*, 2(2), 128–149. <https://doi.org/10.1002/wics.73>

Lenth, R. V. (2009). Response-Surface Methods in R, Using rsm. *Journal of Statistical Software*, 32(7), 1-17. DOI: 10.18637/jss.v032.i07

Capitolo 2

Comparison of the agronomic performance of malting barley cultivars and wheat and rye landraces in a Mediterranean environment (South Italy)

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Abstract

The malting and brewing industry requires raw materials that meet defined quality standards. At the same time, alongside well-established varieties that represent a benchmark for farmers and the malting and brewing industry, there is an increasing focus on old or minor cereal varieties. In the following study, modern varieties of malting barley were compared with Calabrian landraces of wheat and rye. The experiment was conducted in two consecutive growing seasons. The environmental effect had a strong impact on the bio-agronomic traits analyzed, with generally negative consequences. Great variability was identified among the species under study. As expected, barley showed excellent characteristics, especially in terms of yield and number of spikes produced per unit area. Summarily, it is possible to state that the landraces under study, albeit with lower bio-agronomic performance, showed greater stability for the traits examined in the two-year experiment.

Keywords: malting barley; wheat; rye; landrace; bio-agronomic evaluation.

1. Introduction

Barley is a cereal belonging to the *Poaceae* family, *Hordeae* tribe. It is distinguished into two subspecies: *Hordeum vulgare* subsp. *vulgare* and *H. vulgare* susp. *spontaneum*. The former term is used in reference to cultivars, the latter to wild forms (Blattner, 2018). Italy's total barley production (spring and winter cultivars) in the year 2022 was 1,059,803 t. Specifically in the Calabria region, total production amounted to 2,170 t ha⁻¹ in the same year (ISTAT data). Good productions for malting barley were recorded in Italy both in 2021 and in 2022 (6.2 t ha⁻¹ and 6.5 t ha⁻¹ on average, respectively). In the same period, average yields of 6 and 7.8 t ha⁻¹ were recorded in varietal tests conducted in Calabria, making it one of the top ranking region considering the central-southern area and the islands (L'Informatore Agrario data). Barley has been cultivated since ancient times, appreciated for its content in fiber and bioactive compounds. It is used in a variety of food sectors, for the production of baby food, for breakfast in flake form, as whole grain or in floury form (Gupta et al., 2010). Barley is the main cereal for beer production due to its starch, protein and enzyme content, as well as the presence of husks that protect the embryo during malting and aid the brewing filtration process (Rani & Bhardwaj, 2021). Both two-row and six-row varieties are cultivated for malting, although the former is preferred for its special traits such as plumpness and uniform grain size. The suitability of a barley variety for malting also begins with an evaluation in the field. Indeed, barley is intended for malting if it exhibits certain characteristics at harvest, such as ideal protein content, grain uniformity, thousand kernel weight, and so on (Singh et al., 2014). Proper agronomic management is essential to fulfill quality standards for the malting and brewing market. Therefore, seeding density and nitrogen fertilization must be well defined. While high seeding doses lead to a reduction in grain size and protein content, high nitrogen rates adversely affect malting barley quality by increasing the protein percentage and reducing the malt extract potential (Edney et al., 2012). Further undesirable effects, such as reduced yields, may occur with late sowing, also taking into account the effect of the growing environment (O'Donovan et al., 2012). Twenty years of experimentation

in Calabria have resulted in the identification of varieties characterized by high production levels and, above all, excellent malting quality.

Wheat (*Triticum* sp.pl.) is a member of the *Poaceae* family. Three ploidy levels can be identified for the genus: diploid (*T. monococcum*, *T. urartu*); tetraploid (*T. turgidum*, *T. timopheevii*); hexaploid (*T. aestivum*, *T. zhukovskyi*) (Bálint et al., 2000). Although there is a renewed interest in the so-called ancient wheat species (einkorn, emmer, spelt), durum and common wheat make up 5 and 95% of world wheat production, respectively (de Sousa et al., 2021). In Italy, common wheat production for 2021 stood at 3,053,265 t, while durum wheat production was higher at 4,065,006.9 t (ISTAT data). These statistics confirm that wheat is one of the nation's most important crops, an essential element in the production of various traditional baked and pasta products. Nevertheless, wheat is also used for the production of malt and beer, although research has mainly focused on the baking aspects. Thus, wheat with a high protein content may lead to problems during brewing (Faltermajer et al., 2014). At the same time, research is interested in identifying varieties that can perform acceptably in low-input cropping systems. Emmer and spelt varieties have already been tested in a Mediterranean environment with promising results in terms of yield and protein content (De Vita et al., 2007). Similarly, interesting findings concern their use in malting and brewing, both alone and in combination with barley malt (Marconi et al., 2013; Mayer et al., 2011). There is a growing interest in the screening of local varieties of common wheat for malting and brewing. Indeed, studies have been conducted to evaluate the quality traits of different malts made from various Sicilian durum wheat landraces (Alfeo et al., 2018), while the cultivar Senatore Cappelli has been tested for the production of a Sardinian craft beer (Mascia et al., 2014).

Rye (*Secale cereale* L.) is not considered to be among the first cereals cultivated by farmers. Compared to the previous two species, it is more resistant to rigid environments and climates. In fact, there are rye species that can thrive in mountain areas up to 2500 m altitude (Schreiber et al., 2021). About one third of the world's rye production is destined for human consumption, in particular for bread making. This cereal has similarities with wheat from a compositional point of view, although the starch and protein content is lower. In contrast, the dietary fiber content is particularly high, among whose constituents beta-glucans and arabinoxylans are prominent (Jonsson et al., 2018). A significant difference in terms of national production is evident when compared to wheat and barley. Slightly less than 13,000 t have been recorded for the year 2021 in Italy (ISTAT data). In the same year, 4,298 t were harvested in the Calabria region (33% of the national data). Even in the case of rye and in relation to the growing environment, good agronomic practice is necessary to obtain the best results. Good yields can be maintained with low sowing rates and nitrogen doses, with the consequent reduction in plant height and therefore reduced lodging (Noland, 2022).

The craft beer movement in Italy is constantly expanding. New breweries proliferate on the territory with the aim of creating peculiar beers and stand out from the crowd. However, most companies are forced to purchase from specialized suppliers, using raw materials that in most cases come from other countries. The use of local ingredients is a form of claiming one's own identity and is a key element in obtaining characteristic beers in which the territorial imprint is enhanced. Therefore, the aim of this preliminary study was to evaluate the bio-agronomic traits of some varieties of malting barley and landraces of wheat and rye. Over time, modern cereal varieties have gradually replaced local ones with the consequent loss of genetic material that could represent an alternative to high-input crops, in view also of climate change and the increasingly important focus on environmental preservation (Newton et al., 2010). Indeed, attention to climate change is becoming increasingly prominent, bearing in mind that it also impacts on agricultural production. Strategies aimed at minimizing their influence on yields and ensuring food security include more efficient use of water and fertilizers (Wang et al., 2018). The potential of local populations maintained by farmers throughout the year to evolve and adapt to environmental changes, including abiotic and biotic events, can be a solution not to be underestimated (Bocci et al., 2020). The cereal landraces under study were compared in terms of

bio-agronomic traits to understand which ones might lend themselves to further investigation of malting.

2. Materials and methods

2.1. Experimental site and raw material

A two-year field experiment (2019-2020; 2020-2021) was carried out at the experimental station of 'Azienda Regionale per lo Sviluppo dell'Agricoltura Calabrese (ARSAC)' located in San Marco Argentano (Calabria region, southern Italy), in one of the most representative cereal-growing areas north of Cosenza city (39°38'N, 16°13'E, 250 m above sea level). Different varieties of barley, wheat and rye were compared in terms of some bio-agronomic traits (Table 1). All the barley varieties were chosen on the basis of agronomic and qualitative results of long-term varietal comparison experiments carried out in Calabria. Each species was studied independently.

Table 1. Cereal varieties used in the two-year experiments.

Species	Variety	Type	Typical utilization
Barley	Concerto	Cultivar	Malting
	Fortuna	Cultivar	Malting
	Laureate	Cultivar	Malting
	Planet	Cultivar	Malting
	Quench	Cultivar	Malting
	Tea	Cultivar	Zootechnical
Wheat	Mazzancoio	Landrace	Bread-making
	Rosia	Landrace	Bread-making
	Simeto	Cultivar	Bread and pasta production
Rye	Aspromonte	Landrace	Bread-making
	Sila	Landrace	Bread-making
	Pollino	Landrace	Bread-making
	Schlager	Cultivar	Grain, straw, green fodder

2.2. Experimental design and statistical analysis

A randomized block experimental design with four repetitions was adopted. In particular, the following varieties were compared:

- for barley, 5 malting varieties (Concerto, Fortuna, Laureate, Planet, Quench) and 1 for zootechnical use (Tea), as control, chosen on the basis of agronomic and qualitative results of long-term varietal comparison experiments carried out in Calabria.
- for wheat, 2 landraces (Mazzancoio and Rosia) locally used for bread-making and 1 commercial variety (Simeto), as control.
- for rye, 3 landraces from the three "National Parks of Calabria" (Pollino, Sila and Aspromonte) and 1 commercial variety of rye (Schlager), as control.

Data analysis was carried out in Rstudio, R version 4.1.0 (R Core Team, 2021). The analysis of variance (ANOVA) for each agronomic trait was performed independently for each species according to the adopted experimental design. Replication, year and variety, together with their interaction, were considered as source of variability. The significance of main and interaction effects was assessed by means of the F test. Significant differences between the average values of each dependent variable were determined using the Tukey's HSD test for $p \leq 0.05$. Basic functions of Rstudio were used for the analysis of variance (ANOVA); 'ggplot' package was used for data visualization (Wickham, 2016). Principal component analysis (PCA) was used to summarize the information contained in the dataset comprising all species by means of 'FactoMineR' (Le et al.,

2008) and 'factoextra' (Kassambara & Mundt, 2020) packages. As the different dependent variables were measured on different scales, data were scaled to unit variance before running PCA.

2.3. Crop management and measurements

Field experiments were carried out on a flat, loamy-clayey soil with a neutral reaction, in succession to a vetch/oat grassland in the first season and in succession to an Italian ryegrass/berseem clover grassland in the second year. The preparatory work consisted of summer ploughing to a depth of 30 cm and double harrowing before sowing. Fertilization was carried out at the pre-sowing stage with 36 units of N and 92 units of P₂O₅ (200 kg ha⁻¹ of diammonium phosphate) and at the end of tillage with 46 units of N (100 kg ha⁻¹ of urea). Sowing was carried out with a parcel seeder using a programmed sowing density of 300 germinable seeds per m² for barley and 350 for rye and wheat. The sowing dates were different between the two years: 6 December 2019 and 18 December 2020, respectively. In addition, chemical weeding was carried out with a mixture of Axial pronto + Columbus in order to control monocotyledonous and dicotyledonous weeds.

During the crop biological cycle, surveys were carried out on certain bio-morphological traits: earing period was determined when approximately 50% of the plants in the plot had their ears completely outside the flag leaf sheath and it has been expressed as the number of days from the 1st of April (reported in tables and figures as days from 1/4); plant height (cm) was determined from the base of the plant to the apex of the ear, excluding the rests; the number of spikes (n m⁻²) was determined by counting the ears present in two representative strips of the plot of approximately 2 m; at harvesting, the grain yield (t ha⁻¹), the thousand kernel weight (g) and the hectoliter weight (kg hL⁻¹) were determined. Parcel production (kg) was recorded and then converted to t ha⁻¹ and referred to the standard moisture content of 13%, in order to compare productions with different moisture contents. Thousand kernel weight (g) was determined by carrying out three measurements on the plot production sample. Hectoliter weight was determined using a GAC II—Grain Analyzer Computer.



Figure 1. Pictures of the experimental field where the agronomic tests were carried out. From left to right and from top to bottom: barley plots; rye plots; cereal harvest; detail of a spike of Mazzancoio wheat.

3. Results

3.1. Meteorological trend

The temperature and rainfall trend for the two years is shown in Figure 2.

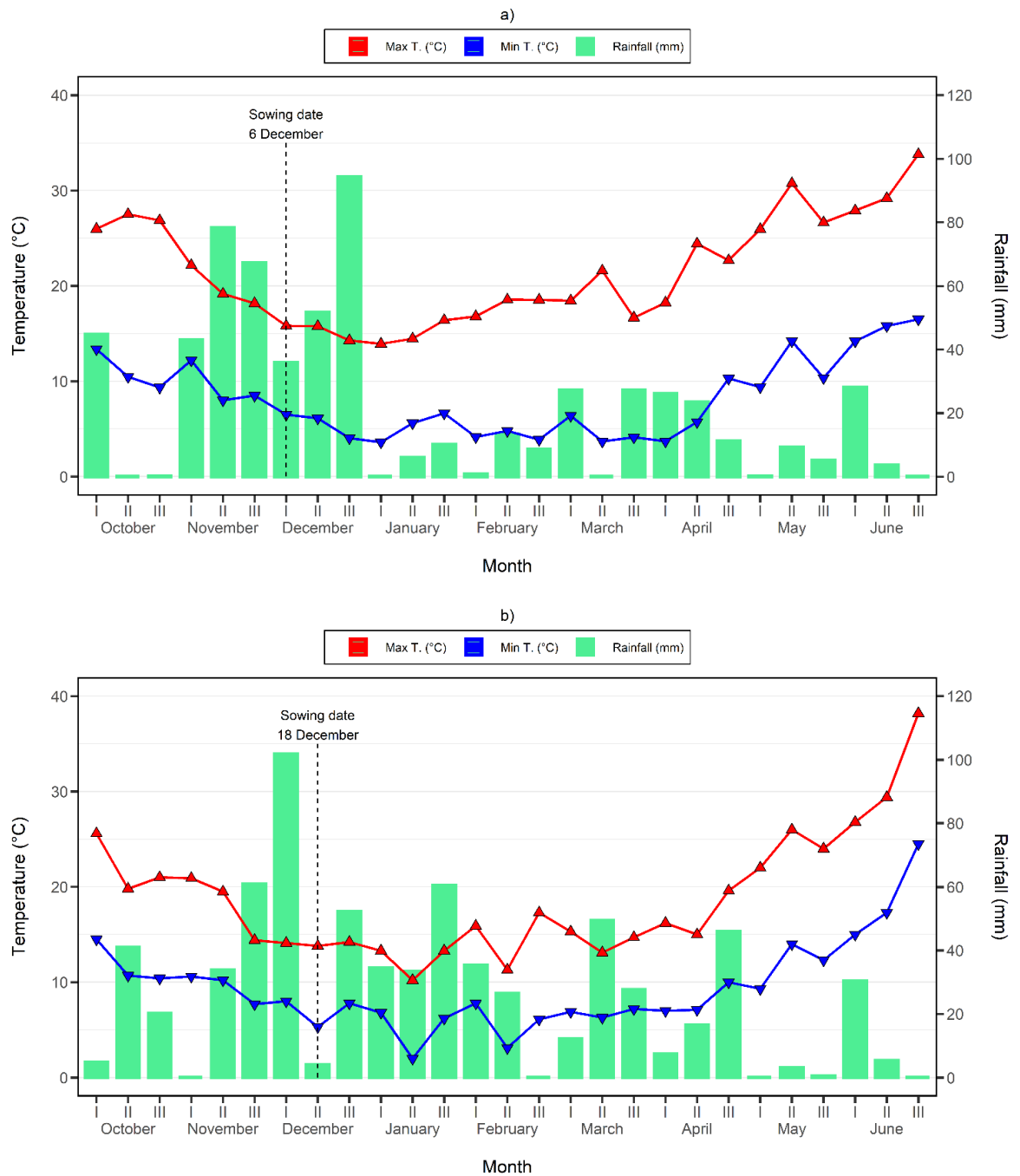


Figure 2. Minimum and maximum temperatures (°C) and rainfall patterns (mm) over the October-June period at the experimental field. The two subplots refer to two different growing seasons: a) 2019/2020; b) 2020/2021.

In the crop season 2019/2020, rainfall amounted to 615 mm in the October-June period; in particular, 38% of the rain fell before sowing (234 mm) and about 30% (182 mm) in the following 20 days, thus reducing the actual total contribution from emergency to harvest to about 200 mm. The low rainfall occurred mainly in the winter period and, in particular, in January and February. Rainfall in March and April provided sufficient water availability for the crops, favoring the phenological phases of rising and earing, with benefits extended to the subsequent graining

phase. Rainfalls recorded between the end of May and the first ten days of June slowed down the ripening of the grains and, consequently, the harvest. As far as the thermal trend is concerned, it should be noted that the winter temperatures have been rather mild with decadal minima never below 0 °C, while a sudden rise above 30 °C only occurred in the third decade of June. In general, despite the difficult weather conditions and the lack of rainfall between January and February, the year was excellent for the cultivation of malting barley.

In the crop season 2020/2021, total rainfall (October–June) reached 706 mm, which was slightly higher than the amount recorded the previous year (615 mm) and the long-term average typical for the area. Before sowing (18/12/2020), the amount of rainfall was 37.7% of the total. Overall, the rainfall pattern was characterized by well-distributed rainfall throughout the period and by temperatures that were generally advantageous for crop development. The continuous heavy rainfall recorded in the second and third decade of November (94.6 mm) and in the first decade of December (101.8 mm) resulted in difficulties in soil preparation. For this reason, sowing was delayed until the end of the second decade of December, when the minimum rainfall for that month was recorded (4.0 mm). Subsequently, in the early stages of the crops biological cycle, modest rainfall recorded between the end of December and February (242 mm) were beneficial. In March, after nitrogen fertilization, there was moderate but well-distributed rainfall, which favored the expression of good vegetative vigor and provided an adequate water supply during the earing phase and then the caryopsis filling phase. However, the delayed sowing time had a negative effect on the bio-morphological behavior of the crop (tillering index, plant height, thousand kernel weight, etc.) with negative consequences on the yield and on the quality traits of the grain. In the first ten days of June, rainfall (30.3 mm) slowed down the senescence phase without, however, damaging the crops. With regard to the temperature trend, it should be noted that winter temperatures were rather mild, with values below 5 °C in the second ten days of January and February. Moreover, sudden temperature rises were recorded in the first ten days of June, reaching a peak of 38.2 °C in the third ten days of the same month.

3.2. Two-row barley

Year exerted a statistically significant effect on the dependent variables, with the sole exception of the hectoliter weight. The variety and the interaction effects were significant for all dependent variables under study (Table 2).

Table 2. Analysis of variance (ANOVA) table for the bio-agronomic traits of barley varieties.

Source of variation	Degree of freedom	F value and significance level of effects					
		Mean squares of Residuals					
		Earing period	Plant height	Spikes	Thousand kernel weight	Hectoliter weight	Yield
Year	1	548.356 ***	102.034 ***	740.914 ***	257.147 ***	3.935	297.092 ***
Variety	5	141.091 ***	55.866 ***	25.656 ***	69.518 ***	95.216 ***	21.110 ***
Replication	3	0.303	2.803	0.830	3.578 *	1.863	0.328
Year x Variety	5	35.337 ***	7.255 ***	5.377 **	12.661 ***	6.726 ***	2.689 *
Residuals	33	0.43	8.3	632	1.4	0.326	0.11

Levels of significance: ‘***’ 0.001 level; ‘**’ 0.01 level; ‘*’ 0.05 level.

Table 3 shows the performance of malting barleys considering only the main effect of the variety.

Table 3. Overall average (\pm sd) of the bio-agronomic trait of barley varieties over the two years.

Variety	Earing period	Plant height	Spikes	Thousand kernel weight	Hectoliter weight	Yield
	(Days from 1/4)	(cm)	(n m ⁻²)	(g)	(kg hL ⁻¹)	(t ha ⁻¹)
Concerto	27 \pm 0.89 ^b	80.7 \pm 2.72 ^b	646 \pm 138.25 ^b	43.3 \pm 3.32 ^c	65.7 \pm 0.91 ^c	7.15 \pm 0.91 ^b
Fortuna	22 \pm 3.62 ^e	75.7 \pm 8.95 ^{cd}	640 \pm 103.83 ^b	48.6 \pm 5.13 ^a	67.2 \pm 0.34 ^b	7.23 \pm 0.84 ^b
Laureate	25 \pm 2.36 ^c	74.2 \pm 2.06 ^{cd}	685 \pm 114.67 ^a	47.6 \pm 4.25 ^{ab}	65.5 \pm 1.07 ^c	7.65 \pm 1.01 ^{ab}
Planet	24 \pm 3.78 ^d	77.7 \pm 7.05 ^{bc}	687 \pm 107.49 ^a	46.5 \pm 3.01 ^b	65.7 \pm 0.84 ^c	8.10 \pm 0.86 ^a
Quench	28 \pm 0.74 ^a	72.9 \pm 3.84 ^d	719 \pm 112.49 ^a	40.1 \pm 2.31 ^d	66.6 \pm 0.67 ^b	7.59 \pm 1.28 ^b
Tea	22 \pm 3.59 ^e	93.6 \pm 7.53 ^a	592 \pm 72.41 ^c	48.6 \pm 0.97 ^a	70.7 \pm 0.55 ^a	6.50 \pm 0.7 ^c

Different letters indicate significant differences between treatments (Tukey's HSD test).

The earing period varied between 22 and 28 days for Fortuna and Quench, respectively. No statistical differences were found for Tea and Fortuna. All varieties were characterized by a plant height lower than 100 cm. In particular, Tea showed an average height of 93.6 cm, while there was a clear discrepancy between Concerto (80.7 cm) and Quench (72.9 cm). The number of spikes m⁻² ranged from 592 to 719 for Tea and Quench, respectively. No statistically significant differences were found for Laureate, Planet and Quench. The same could be stated for Concerto and Fortuna, which formed a distinct group. Thousand kernel weight ranged from 40.1 to 48.6 g. Tea and Fortuna showed the same value on average (48.6 g). Intermediate values were measured for Laureate, Planet and Concerto. Tea showed also the greater value for the hectoliter weight (70.7 kg hL⁻¹). Fortuna and Quench were characterized by the highest values among the malting barley varieties (67.2 and 66.6 kg hL⁻¹, respectively), whose differences were minimal, overall. Planet resulted the most productive among the varieties, reaching a mean yield of 8.10 t ha⁻¹. Yields for the other malting varieties ranged between 7.15 and 7.65 t ha⁻¹ for Concerto and Laureate, respectively.

In general, with the exception of the earing period and the hectoliter weight, there was a decrease in the mean values of each examined dependent variable (Figure 3). The earing period varied from 18 to 28 days in the first year, from 25 to 29 in the second year. No significant differences were found between the two years for Concerto and Quench. On the contrary, a significant delay was observed for the remaining varieties if compared to the first year (18 to 25 days for Fortuna, 23 to 27 for Laureate, 20 to 27 for Planet, 19 to 25 for Tea). The plant height was less than 1 meter considering all varieties. There was a decrease in the variable between the two growing seasons: from 75.4 to 99.9 cm in the first year, from 68.4 to 87.4 cm in the second year. Tea proved to be the largest size variety. The difference in the number of spikes m⁻² was significant among the two years (760 and 563 n m⁻² on average, respectively) and this influenced the crop's production behavior, which was significantly higher in the first year of the trial. Among the malting barleys, the number of spikes m⁻² varied from 730 to 824 for Fortuna and Quench in the first year, and from 517 to 615 for Concerto and Quench in the second year, respectively. Regarding the thousand kernel weight, Tea resulted the only variety that showed stability over the two periods. There was a significant decrease for the remaining varieties. No statistically significant differences were identified between Fortuna and Laureate within the first year. They showed the highest values of 53.3 and 51.5 g, respectively. The following year, however, the two just mentioned varieties showed a thousand kernel weight similar to that of Planet (44.1 g). On the whole, all the varieties under study showed an almost constant hectoliter weight. Tea showed the greater value among the two years (>70 kg hL⁻¹). All the malting varieties showed values below 70 kg hL⁻¹. In particular, no difference was detected for the Fortuna variety among the two years. Malting barleys gave higher yields than the Tea variety. In the first year the values varied in the range 7.97–8.88 t ha⁻¹ for Fortuna and Planet, respectively. In the second year, lower yields in the range 6.31–7.32 t ha⁻¹ were recorded for Concerto and Planet, respectively. Finally, Laureate, Planet and Quench were the most productive malting barley varieties.

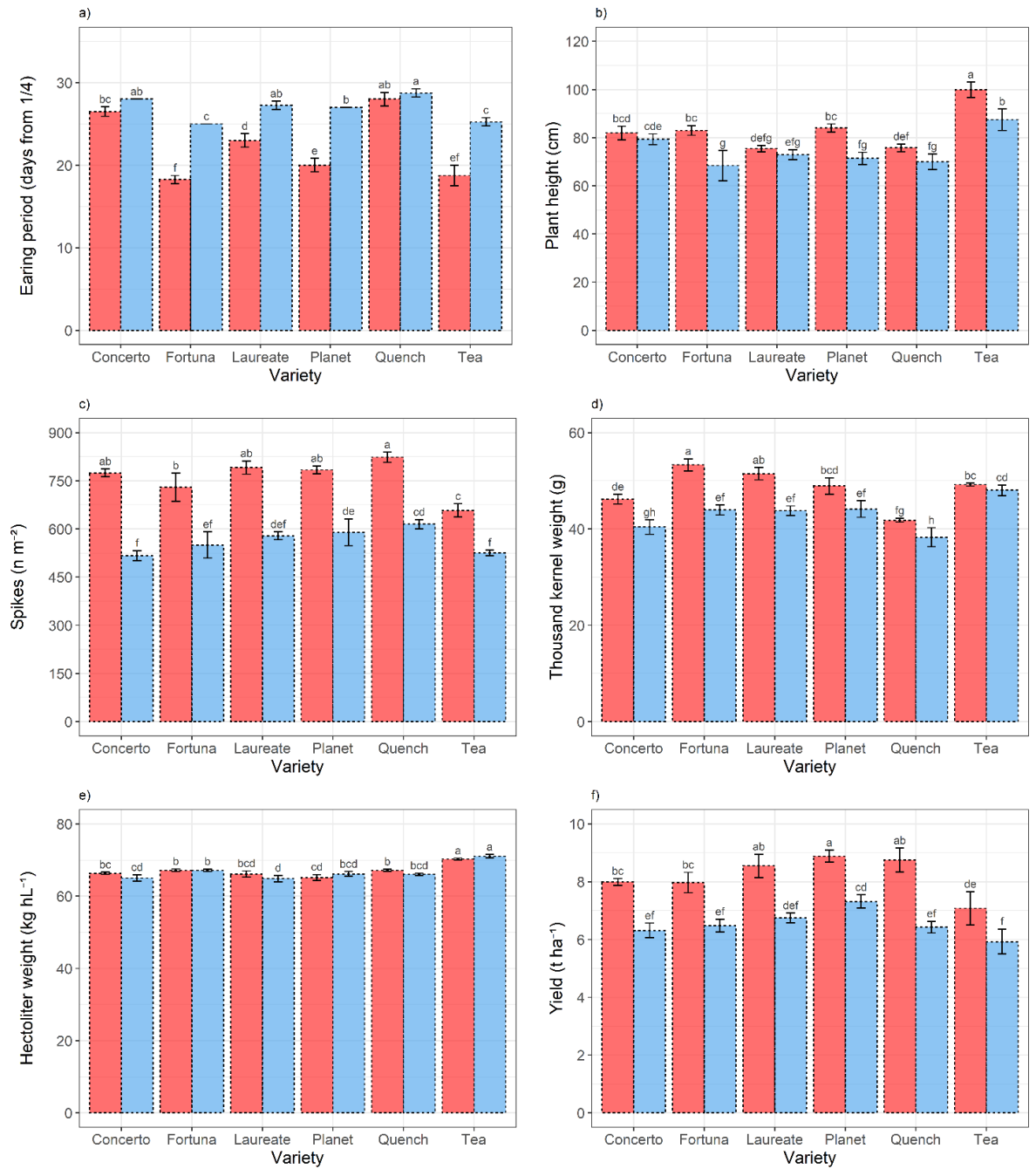


Figure 3. Comparison of mean values for each bio-agronomic trait considering barley varieties and crop season interaction: a) Earing period; b) Plant height; c) Spikes; d) Thousand kernel weight; e) Hectoliter weight; f) Yield. The different colors refer to different crop seasons: red for 2019/2020, blue for 2020/2021. Within the same graph, different letters indicate significant differences between treatments (Tukey's HSD test).

3.3. Wheat

The bio-agronomic performances of the three wheat genotypes resulted different, with the landraces group (Mazzancoio e Rosia) distinguished from the commercial variety (Simeto). The year-by-variety interaction on all variables considered was also significant (Table 4).

Table 4. Analysis of variance (ANOVA) table for the bio-agronomic traits of wheat varieties.

Source of variation	Degree of freedom	F value and significance level of effects					
		Mean squares of Residuals					
		Earing period	Plant height	Spikes	Thousand kernel weight	Hectoliter weight	Yield
Year	1	28.256 ***	11.296 **	6.279 *	163.550 ***	24.675 ***	25.272 ***
Variety	2	4632.209 ***	1605.747 ***	17.616 ***	86.608 ***	25.819 ***	335.557 ***
Replication	3	6.860 **	1.742	0.883	0.679	0.692	0.242
Year x Variety	2	7.326 **	10.277 **	3.722 *	8.947 **	17.273 ***	2.161
Residuals	15	0.1	13	763	2.0	0.294	0.055

Levels of significance: '***' 0.001 level; '**' 0.01 level; '*' 0.05 level.

Mean values for each bio-agronomic trait for the wheat genotypes are reported in Table 5.

Table 5. Overall average (\pm sd) of the bio-agronomic and quality traits of wheat varieties over the two years.

Variety	Earing period	Plant height	Spikes	Thousand kernel weight	Hectoliter weight	Yield
	(Days from 1/4)	(cm)	(n m ⁻²)	(g)	(kg hL ⁻¹)	(t ha ⁻¹)
Mazzancoio	39 \pm 0.74 ^b	171.8 \pm 2.40 ^a	289 \pm 17.78 ^c	53.4 \pm 5.72 ^b	80.4 \pm 0.78 ^a	3.89 \pm 0.30 ^b
Rosia	41 \pm 0.76 ^a	154.7 \pm 8.88 ^b	328 \pm 53.24 ^b	48.3 \pm 4.20 ^c	78.9 \pm 1.53 ^b	3.38 \pm 0.23 ^c
Simeto	25 \pm 0.46 ^c	77.3 \pm 3.06 ^c	371 \pm 16.32 ^a	57.6 \pm 2.61 ^a	78.6 \pm 0.50 ^b	6.23 \pm 0.46 ^a

Different letters indicate significant differences between treatments (Tukey's HSD test).

The earing period was in the range 25–41 days for Simeto and Rosia, respectively. All three genotypes presented significantly different timeframes, extending into May for wheat landraces, indicating a late biological cycle compared to the early-cycle Simeto. The plant height was found to be significantly divergent between the Simeto variety and the two landraces. The latter, in fact, were characterized by heights clearly over 150 cm. In particular, Rosia presented an average height of 154.7 cm, while Mazzancoio was the tallest variety, with an average value of 171.8 cm. The number of spikes m⁻² varied between 289 and 371 for Mazzancoio and Simeto, respectively. The grains of the Simeto variety showed the highest thousand kernel weight on average (57.6 g), while the two landraces presented lower values (53.4 g for Mazzancoio and 48.3 g for Rosia). Mazzancoio showed the highest hectoliter weight (80.4 kg hL⁻¹), while there was no statistically significant difference between Rosia and Simeto (78.9 and 78.6 kg hL⁻¹, respectively). The productivity of Simeto (6.23 t ha⁻¹) was significantly higher than that of the two populations of Rosia and Mazzancoio (3.38 and 3.89 t ha⁻¹, respectively).

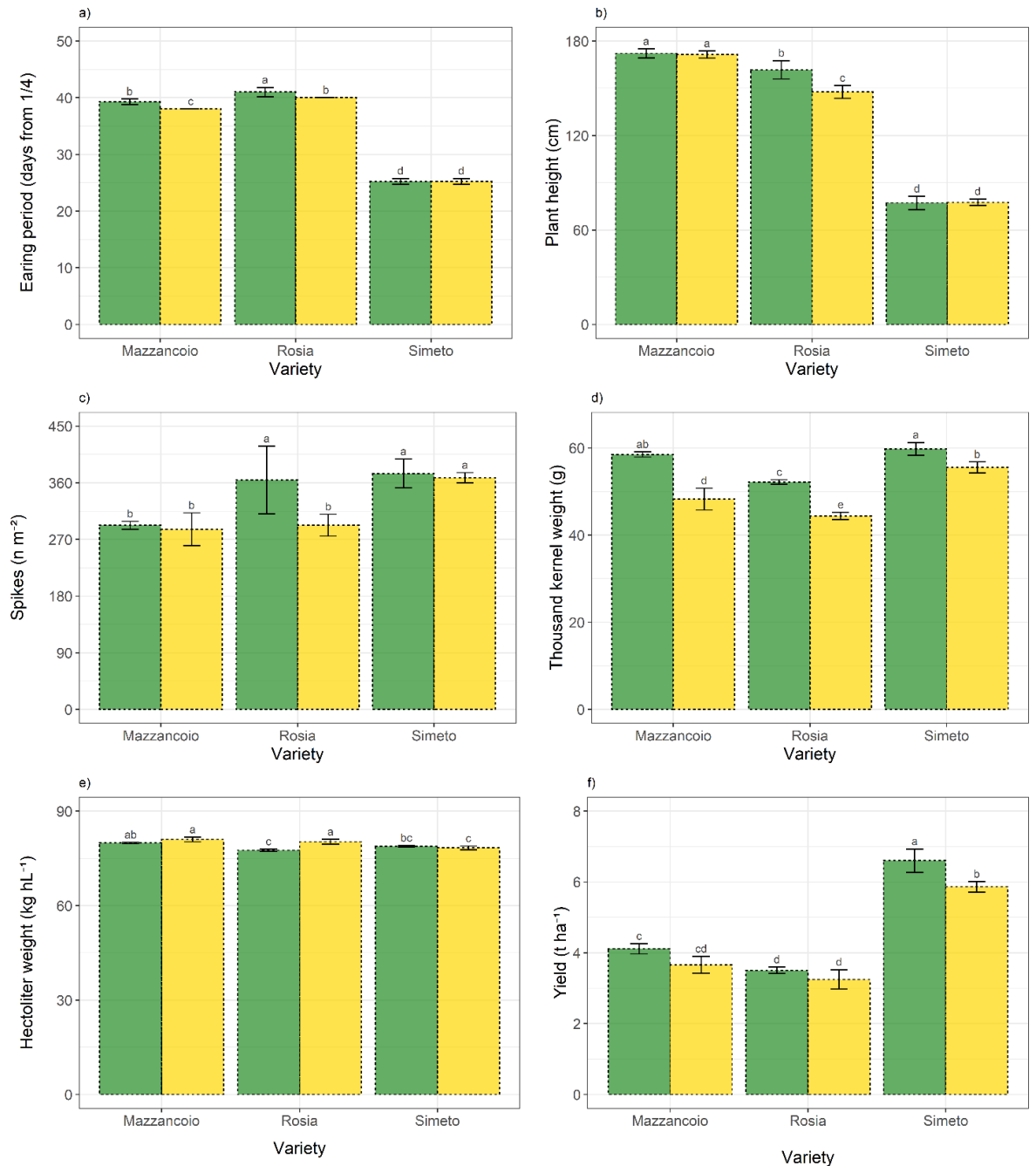


Figure 4. Comparison of mean values for each bio-agronomic trait considering wheat varieties and crop season interaction: a) Earing period; b) Plant height; c) Spikes; d) Thousand kernel weight; e) Hectoliter weight; f) Yield. The different colors refer to different crop seasons: green for 2019/2020, yellow for 2020/2021. Within the same graph, different letters indicate significant differences between treatments (Tukey's HSD test).

The earing period was similar among the two years and the varieties under study (Figure 4). In particular, it resulted the same for Simeto in both growing seasons (25 days), while for Mazzancoio and Rosia there was only 1 day of difference comparing the two years (39–38 days for Mazzancoio and 41–40 for Rosia, respectively). In the first year of cultivation, the average plant height varied from 77.2 cm to 172.2 cm for Simeto and Mazzancoio, respectively. In the second year, heights remained approximately the same for the two varieties just mentioned, while a significant decrease was found for Rosia, whose average height dropped by approximately 14 cm. The same trend was observed regarding the number of spikes m⁻² among the first and second year. In particular, Simeto was confirmed as the variety with the highest

spikes production, with a non-significant decrease between the two growing seasons (375–368 number of spikes m⁻² in the first and second year, respectively). No change was also detected for Mazzancoio, which still ranked last. On the other hand, Rosia was probably influenced by the climatic conditions in the test environment, as there was a decrease in the number of spikes produced, from 364 to 293. There was a significant drop in thousand kernel weight among the two years for all wheat varieties. The values for the first growing season varied from 52.2 g to 59.8 g for Rosia and Simeto, respectively. Subsequently, the range of measured values varied from 44.4 to 55.5 for the same varieties mentioned above. It is interesting to note that in the first year, the thousand kernel weight for Simeto and Mazzancoio was similar and close to 60 g. The hectoliter weight was roughly stable between the test years and similar among the different varieties. It ranged from 77.6 to 79.9 kg hL⁻¹ in the first cropping season, while in the following one values between 78.3 and 81.0 kg hL⁻¹ were recorded. Simeto was definitely the best performing variety in terms of yield, with crop productions varying between 6.60 and 5.86 t ha⁻¹ between the first and second year. This was followed by Mazzancoio and Simeto, whose average yields were less than 4.2 t ha⁻¹.

3.4. Rye

The year and variety effects resulted significant for all observed traits. Only plant height was not affected by the interaction between year and variety (Table 6).

Table 6. Analysis of variance (ANOVA) table for the bio-agronomic traits of rye varieties.

Source of variation	Degree of freedom	F value and significance level of effects					
		Mean squares of Residuals					
		Earing period	Plant height	Spikes	Thousand kernel weight	Hectoliter weight	Yield
Year	1	43.615 ***	27.888 ***	37.394 ***	81.513 ***	12.834 **	38.211 ***
Variety	3	222.744 ***	29.710 ***	30.115 ***	35.281 ***	42.611 ***	24.958 ***
Replication	3	0.897	1.182	0.064	0.617	2.977	6.496 **
Year x Variety	3	126.538 ***	2.721	9.244 ***	3.081 *	15.770 ***	3.397 *
Residuals	21	0.23	28.7	370	2.06	0.396	0.0431

Levels of significance: '***' 0.001 level; '**' 0.01 level; '*' 0.05 level.

The earing period resulted significantly different for the varieties under study (Table 7). Sila and Schlager showed the same result of 34 days, while Pollino turned out to be the earliest. All genotypes were characterized by tall culms, for values above 150 cm. In particular, the plant height ranged from 154.7 to 177.0 cm on average for Aspromonte e Pollino landraces. The number of spikes was not statistically different for Aspromonte e Sila with an average value of 352 spikes m⁻². Pollino and Schlager were characterized by a lower spike density. Thousand kernel weight varied from 25.9 g for Sila to 32.4 g for Pollino. The hectoliter weight did not varied considerably, although significant differences could be detected between the landraces under study. Pollino presented the highest value of 75.5 kg hL⁻¹. Aspromonte resulted the most productive variety in terms of grain yield, with an average value of 4.35 t ha⁻¹. The other varieties presented yield below 4 t ha⁻¹, with values ranging from 3.52 to 3.80 t ha⁻¹ for Schlager and Sila, respectively.

Table 7. Overall average (\pm sd) of the bio-agronomic and quality traits of rye varieties over the two years.

Variety	Earing period	Plant height	Spikes	Thousand kernel weight	Hectoliter weight	Yield
	(Days from 1/4)	(cm)	(n m ⁻²)	(g)	(kg hL ⁻¹)	(t ha ⁻¹)
Aspromonte	33 \pm 0.76 ^b	154.7 \pm 7.31 ^c	361 \pm 55.55 ^a	31.6 \pm 1.92 ^a	74.5 \pm 2.00 ^b	4.35 \pm 0.47 ^a
Sila	34 \pm 3.36 ^a	156.5 \pm 10.39 ^b	343 \pm 29.59 ^a	25.9 \pm 2.81 ^c	73.0 \pm 0.46 ^c	3.80 \pm 0.46 ^b
Pollino	29 \pm 1.69 ^c	177.0 \pm 6.24 ^a	294 \pm 22.22 ^b	32.4 \pm 3.49 ^a	75.5 \pm 0.76 ^a	3.63 \pm 0.27 ^b
Schlager	34 \pm 0.35 ^a	166.9 \pm 6.92 ^b	283 \pm 10.65 ^b	28.1 \pm 3.10 ^b	72.2 \pm 0.57 ^c	3.52 \pm 0.21 ^b

Different letters indicate significant differences between treatments (Tukey's HSD test).

The earing period varied between 30 to 34 days in the first year for Pollino and Schlager varieties, respectively; in the second year it ranged between 27 to 37 days for Pollino and Sila, respectively (Figure 5). It was possible to identify a particular pattern between the two growing seasons for the different varieties. Thus, while the earing period tended to increase from the first to the second year for Aspromonte, Sila and Schlager, there was at the same time a decrease for Pollino. The commercial variety was also stable over time. All genotypes were characterized by plant heights close to or above 150 cm. The second season saw increases in plant size, although only for Sila this phenomenon was statistically different. In the second year, heights varied from 160.2 cm for Aspromonte to 179.4 cm for Pollino. Aspromonte and Sila were significantly influenced by the interaction effect of year and variety on the quantity of spikes m⁻². Both landraces produced fewer spikes in the second year, although for Sila this decrease was of minor magnitude. On the contrary, Pollino and Schlager presented a lower number of spikes, that remained stable among the two growing seasons. In particular, while in the first year it was possible to distinguish two groups, with Aspromonte and Sila proving to be the most productive in terms of quantity of spikes produced (409 and 368, respectively), in the second year no statistically significant difference was noticed, with values varying from 276 for Schlager to 319 for Sila. The thousand kernel weight varied from 28.5 g for Sila to 35.4 g for Pollino in the first year; it ranged from 23.4 g for Sila to 30.6 g for Aspromonte in the second year. Overall, there was a decrease in the thousand kernel weight for all varieties. On the other hand, the hectoliter weight resulted almost unaffected among the two years, with values comprised between 72.0 and 76.2 kh hL⁻¹ for Schlager and Aspromonte, respectively. Average yields were also affected by the interaction effect, generally being lower in the second year. In both growing seasons, the most productive variety proved to be Aspromonte (4.63 and 4.06 t ha⁻¹ in the first and second year, respectively). The lowest yields were recorded for Schlager in both the first and second year (3.65 and 3.39 t ha⁻¹, respectively).

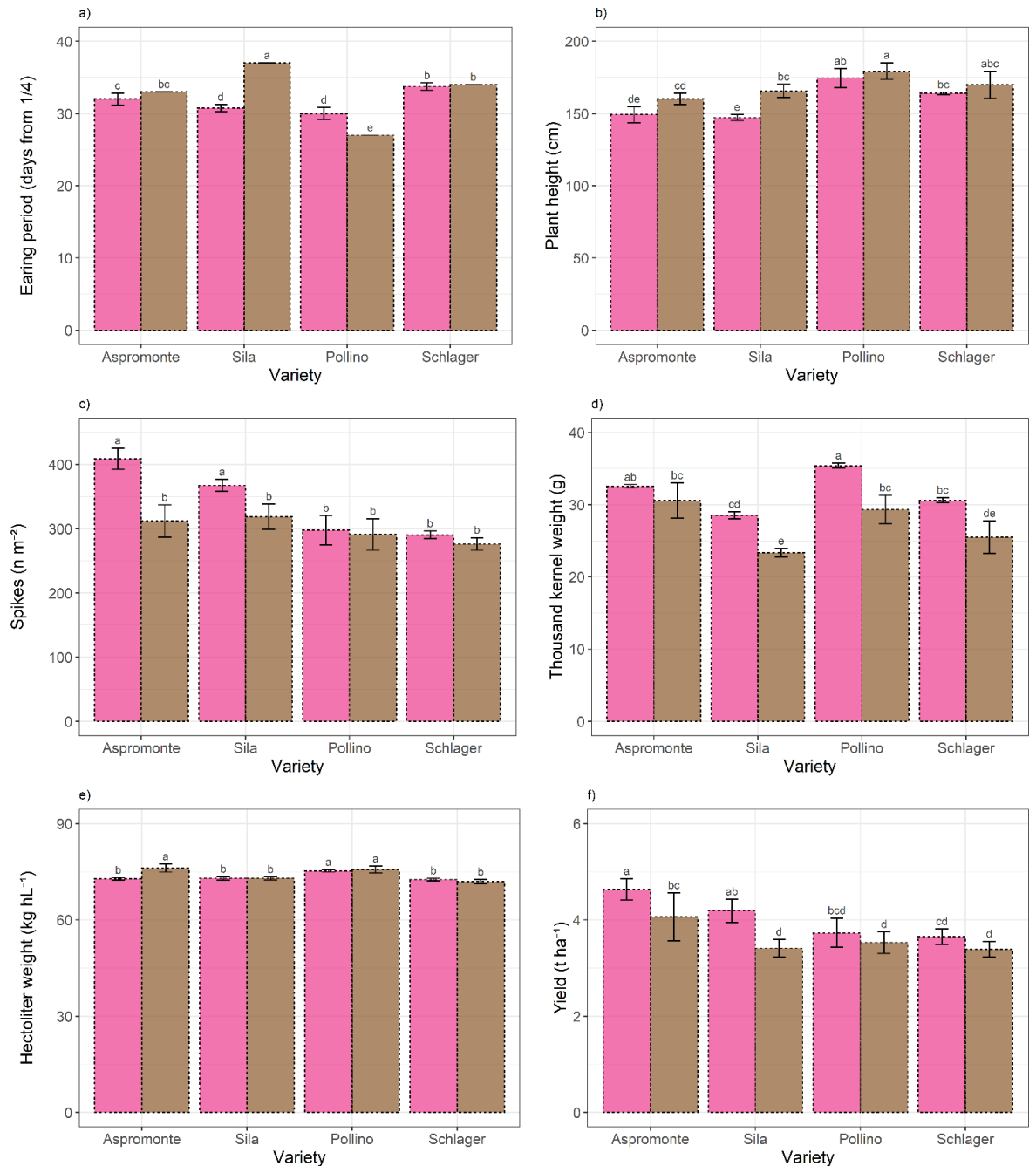


Figure 5. Comparison of mean values for each bio-agronomic trait considering rye varieties and crop season interaction: a) Earing period; b) Plant height; c) Spikes; d) Thousand kernel weight; e) Hectoliter weight; f) Yield. The different colors refer to different crop seasons: violet for 2019/2020, tan for 2020/2021. Within the same graph, different letters indicate significant differences between treatments (Tukey's HSD test).

4. Discussion

The earing date is a cereal development stage that coincides with the appearance of the spike from the flag leaf. This stage is considered important in terms of yield and environmental adaptability, particularly regarding barley breeding (Alqudah & Schnurbusch, 2017). For barley varieties, the earing period varied from 22 to 27 days, with the lowest mean value recorded for Tea. Among the varieties tested in the two years, Quench and Concerto presented the greatest stability (28–29 and 27–28 days for the two varieties in the first and second year, respectively), while for the others the earing period varied significantly. Indeed, spike emergence was delayed in the second year as a consequence of late sowing. In the case of wheat and rye, there was less

variability, albeit it resulted significant within the same varieties between the two crop seasons. In particular, when comparing the different species under study, the rye variety Schlager did not vary in the test area. Overall, wheat and rye required a longer development time, ranging from 39 to 41 days and from 29 to 34 days for wheat and rye landraces, respectively. A clear discrepancy could be seen between the two landraces and the control variety of wheat, whose earing period was considerably shorter. The plant height for domesticated cereals is a character that has been modified over time as a consequence of breeding programs, with the aim of reducing plant size. This resulted in a lower risk of lodging with consequent advantages in terms of more effective mechanized harvesting (Niu et al., 2021). High discrepancy was found among the species. The control variety Tea presented the highest plant heights among the barleys varieties. Laureate and Planet showed similar results in the two years, although overall, the environmental effect led to a decrease in average heights, particularly in the case of Fortuna and Planet. On the other hand, wheat and rye were characterized by considerable development in terms of culm length. Similar plant heights characterized the local varieties, with values above 150 cm. As a matter of fact, landraces tend to reach considerable heights as this character has not been modified by genetic improvement. For this reason, they are generally more prone to allurement with consequent damages at harvest time, especially under adverse climatic conditions. While the difference in height values was not remarkable among the rye varieties, it is worth noting the presence of two distinct groups for wheat, with the Simeto variety presenting an average height of 77.4 cm in the two years, while the landraces presented heights close to 180 cm in the first year. The number of spikes m^{-2} is one of the main components determining cereal production. Malting barleys presented the highest spikes density, ranging from 640 to 719 spikes m^{-2} . They produced more spikes m^{-2} than Tea, although the differences were less marked in the second crop season. This could be explained by a greater susceptibility of the former to weather variations. Significantly lower results has been recorded for the landraces, with values around or below to 400 spikes m^{-2} . In particular, Mazzancoio and Pollino showed the greatest stability over the time period examined. Based on the average of the two years, the number of spikes m^{-2} ranged between 289 and 371 for wheat and from 283 to 361 for rye, with the minimum value recorded for the commercial variety Schlager. In addition, both control varieties (Simeto and Schlager) for the two species just mentioned kept the number of spikes produced in the two growing seasons almost unchanged, thus showing good stability. In general, however, it could be observed that the effect of the environment was preponderant in determining a drastic drop in the number of ears, and consequently in terms crop yield, in the second year. The thousand kernel weight was found to vary widely between species. Also in this case, the environment exerted a significant effect with lower values in the second cropping season for all the species. In the case of barley, between the two seasons it was however possible to notice the same trend, with Fortuna, Laureate and Planet which stood out for the higher values of thousand kernel weight. For Quench, on the contrary, the lower weights were recorded, while Tea has kept this qualitative characteristic almost unchanged. Both in the case of barley and wheat, the range of values for the thousand kernel weight resulted higher than rye (38.3–53.3 g, 44.4–59.8 g and 25.9–32.4 g, respectively). The hectoliter weight was the variable that varied the least within species and between years. Also in this case, wheat excelled with values close to 80 kg hL^{-1} , followed by rye (72.2–75.5 kg hL^{-1}) and then barley varieties 65.5–70.7 kg hL^{-1}), considering the results over the two years. Yield is a character influenced by several factors, most notably cultivar and nitrogen use efficiency. Increasing yields over the years have been shown to be correlated with a decrease in the protein content of the caryopses (Guarda et al., 2004). Traits positively correlated with yield in the case of barley are, among others, plant height and thousand kernel weight (Adhikari & Shrestha, 2018). As expected, barley yields were remarkably high. The varieties under study, in fact, allow to obtain good productions by adopting a suitable agronomic technique. While barley yield averaged 7.54 t ha^{-1} , wheat and rye landraces recorded average productions of 3.64 and 3.93 t ha^{-1} , respectively, clearly below that observed for the species mentioned above. This is not surprising, as it is a comparison between varieties with different basic performances.

Nevertheless, the yields of Rosia and Pollino were not significantly different between the two seasons.

To obtain an overall understanding of the variables affecting the species under study, a PCA was performed on the original dataset considering the two test years together and excluding observations on control varieties. Hence, the PCA was carried out on 80 observations, that were described by the following 6 variables: EP (earring period), PH (plant height), NS (number of spikes), TKW (thousand kernel weight), HW (hectoliter weight), Y (yield). Six principal components or dimensions were obtained from the PCA. The first two components explained 91.7% of the total variance (74.6% by component 1 and 17.1% by component 2). The remaining components explained a total variance of 8.3% (Figure 6a). Taking this into account, only the first two components have been retained. The most important variables in the determination of the principal components resulted TKW and HW, whose total contribution to PC1 and PC2 was 86.4% and 27.2%, respectively (Figure 6b). The variable correlation plot (Figure 6c) depicts the relationship between all variables under study. Overall, all variables are well represented on the factor map, their vectors being more or less close to the circumference and far from the origin. TKW was very important in interpreting principal component 2; HW and EP stood between the two components; the remaining variables were very important in interpreting principal component 1. The representation of EP variable was the lowest. The variables EP, PH and HW were positively correlated with PC1, while NS, TKW and Y were negatively correlated; moreover, there was a positive correlation between EP, NS, TKW, HW, Y and PC2, while PH resulted negatively correlated (Table 8). As shown in Figure 6d, three different groups have been identified according to the species they belong to. In particular, observations for barley presented high values for yield and number of spikes m⁻²; observations for wheat were mainly characterized by high values for hectoliter weight and earring period; in the case of rye, observations presented high values especially regarding plant height.

Table 8. Correlation coefficients of variables and principal components 1 and 2.

Variable	Correlation	
	PC1	PC2
EP	0.85	0.28
PH	0.93	-0.01
NS	-0.96	0.08
TKW	-0.34	0.93
HW	0.93	0.29
Y	-0.98	0.09

EP, earring period; PH, plant height; NS, number of spikes, TKW, thousand kernel weight; HW ,hectoliter weight; Y, yield.

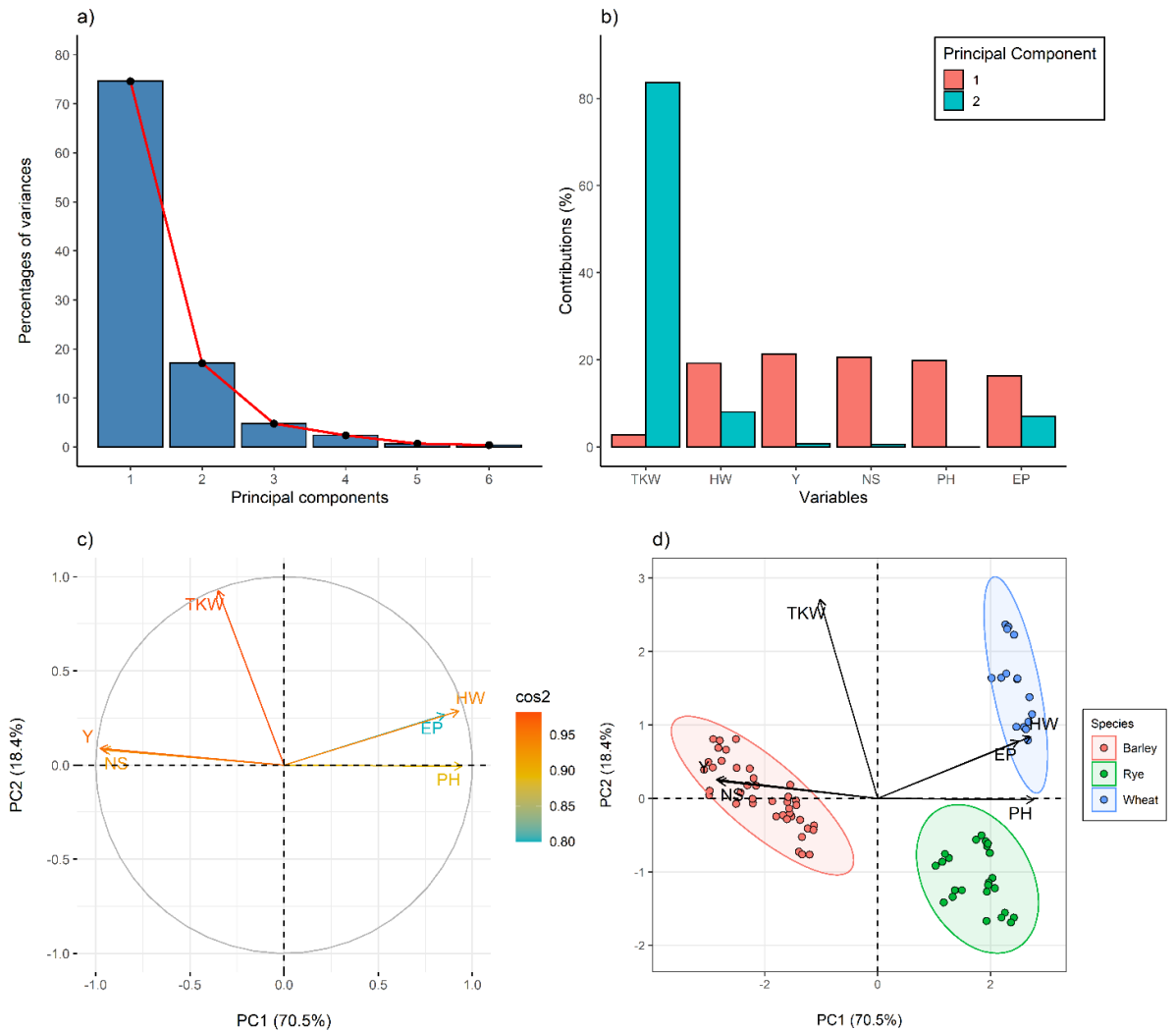


Figure 6. Principal component analysis plots: a) Scree plot of eigenvalues/variances explained by each principal component; b) Variables contribution to PC1 (pink) and PC2 (blue); c) Variable correlation plot: variable vectors present different colors based on their specific quality of representation (\cos^2), as depicted in the legend; d) PCA biplot of individuals and variables. EP, earing period; PH, plant height; NS, number of spikes, TKW, thousand kernel weight; HW, hectoliter weight; Y, yield.

5. Conclusions

This study compared the agronomic performances of modern malting barley varieties and landraces of wheat and rye, typically grown and maintained by local farmers, in a two-year experiment in Calabria region, South Italy. On the whole, the interaction effect between genotypes and test year was significant and resulted in a decrease for most of the bio-agronomic traits evaluated. As expected, malting barley showed excellent agronomic performance in the test environment, confirming that quality production for the malt industry is feasible in southern Italy. Landraces were characterized by high plant heights, higher hectoliter weights and longer earing periods when compared to barley. Grain yields for the landraces were lower than for barley, but the gap between the two growing seasons was greater for the latter species, confirming a greater ability of old populations to mitigate environmental effects. Further studies are needed to identify the best agronomic management for the species under study. In addition, further analyses of the grain obtained from the old populations are moreover required in order to assess their suitability for malting and to identify which would be best suited to this process.

References

1. Blattner, F. R. (2018). Taxonomy of the Genus *Hordeum* and Barley (*Hordeum vulgare*). In N. Stein & G. J. Muehlbauer (A c. Di), *The Barley Genome* (pagg. 11–23). Springer International Publishing. https://doi.org/10.1007/978-3-319-92528-8_2
2. ISTAT, <http://dati.istat.it/> (21 September 2022)
3. L'Informatore Agrario, <https://www.informatoreagrario.it/> (22 September 2022)
4. Gupta, M., Abu-Ghannam, N., & Gallagher, E. (2010). Barley for Brewing: Characteristic Changes during Malting, Brewing and Applications of its By-Products. *Comprehensive Reviews in Food Science and Food Safety*, 9(3), 318–328. <https://doi.org/10.1111/j.1541-4337.2010.00112.x>
5. Rani, H., & Bhardwaj, R. D. (2021). Quality attributes for barley malt: “The backbone of beer”. *Journal of Food Science*, 86(8), 3322–3340. <https://doi.org/10.1111/1750-3841.15858>
6. Singh, A., Singh, H., Kang, J. S., & Singh, J. (2014). REVIEW ARTICLE ADVANCEMENT OF AGRONOMIC PRACTICES IN MALTING BARLEY-A REVIEW. *International Journal of Current Research*, 6(02), 4921-4935.
7. Edney, M. J., O'Donovan, J. T., Turkington, T. K., Clayton, G. W., McKenzie, R., Juskiw, P., Lafond, G. P., Brandt, S., Grant, C. A., Harker, K. N., Johnson, E., & May, W. (2012). Effects of seeding rate, nitrogen rate and cultivar on barley malt quality: Effects of seeding rate, nitrogen rate and cultivar on barley malt quality. *Journal of the Science of Food and Agriculture*, 92(13), 2672–2678. <https://doi.org/10.1002/jsfa.5687>
8. O'Donovan, J. T., Turkington, T. K., Edney, M. J., Juskiw, P. E., McKenzie, R. H., Harker, K. N., Clayton, G. W., Lafond, G. P., Grant, C. A., Brandt, S., Johnson, E. N., May, W. E., & Smith, E. (2012). Effect of seeding date and seeding rate on malting barley production in western Canada. *Canadian Journal of Plant Science*, 92(2), 321–330. <https://doi.org/10.4141/cjps2011-130>
9. Bálint, A. F., Kovács, G., & Sutka, J. (2000). ORIGIN AND TAXONOMY OF WHEAT IN THE LIGHT OF RECENT RESEARCH. *Acta Agronomica Hungarica*, 48(3), 301–313. <https://doi.org/10.1556/AAgr.48.2000.3.11>
10. de Sousa, T., Ribeiro, M., Sabeça, C., & Igrejas, G. (2021). The 10,000-Year Success Story of Wheat! *Foods*, 10(9), 2124. <https://doi.org/10.3390/foods10092124>
11. Faltermaier, A., Waters, D., Becker, T., Arendt, E., & Gastl, M. (2014). Common wheat (*Triticum aestivum* L.) and its use as a brewing cereal - a review: Common wheat and its use as a brewing cereal. *Journal of the Institute of Brewing*, 120(1), 1–15. <https://doi.org/10.1002/jib.107>
12. De Vita, P., Mastrangelo, A. M., Codianni, P., & Fornara, M. (2007). Bio-agronomic Evaluation of Old and Modern Wheat, Spelt and Emmer Genotypes for Low-input Farming in Mediterranean Environment. *Italian Journal of Agronomy*, 2(3), 291. <https://doi.org/10.4081/ija.2007.291>
13. Marconi, O., Mayer, H., Chiacchieroni, F., Ricci, E., Perretti, G., Fantozzi P. “The influence of glumes on malting and brewing of hulled wheats” *Journal of the American Society of Brewing Chemists*, 71(1), (2013) 41-48.
14. Mayer, H., Marconi, O., Perretti, G., Sensidoni, M., Fantozzi, P. “Investigation on suitability of hulled wheats for malting and brewing” *Journal of the American Society of Brewing Chemists* 69, 2, (2011) 116-120.
15. Alfeo, V., Jaskula-Goiris, B., Venora, G., Schimmenti, E., Aerts, G., & Todaro, A. (2018). Screening of durum wheat landraces (*Triticum turgidum* subsp. *Durum*) for the malting suitability. *Journal of Cereal Science*, 83, 101–109. <https://doi.org/10.1016/j.jcs.2018.08.001>
16. Mascia, I., Fadda, C., Dostálek, P., Olšovská, J. and Del Caro, A. (2014), Preliminary characterization of an Italian craft durum wheat beer, *J. Inst. Brew.*, 120, 495– 499. <https://doi.org/10.1002/jib.176>
17. Schreiber, M., Özkan, H., Komatsuda, T., Mascher, M. (2021). Evolution and Domestication of Rye. In: Rabanus-Wallace, M.T., Stein, N. (eds) *The Rye Genome. Compendium of Plant Genomes*. Springer, Cham. https://doi.org/10.1007/978-3-030-83383-1_6
18. Jonsson, K., Andersson, R., Bach Knudsen, K. E., Hallmans, G., Hanhineva, K., Katina, K., Kolehmainen, M., Kyrø, C., Langton, M., Nordlund, E., Lærke, H. N., Olsen, A., Poutanen, K., Tjønneland, A., & Landberg, R. (2018). Rye and health—Where do we stand and where do we go? *Trends in Food Science & Technology*, 79, 78–87. <https://doi.org/10.1016/j.tifs.2018.06.018>
19. Noland, R. L. (2022). Rye grain response to nitrogen fertilizer and seeding rate. *Agrosystems, Geosciences & Environment*, 5(1). <https://doi.org/10.1002/agg2.20239>

20. Newton, A. C., Akar, T., Baresel, J. P., Bebeli, P. J., Bettencourt, E., Bladenopoulos, K. V., Czembor, J. H., Fasoula, D. A., Katsiotis, A., Koutis, K., Koutsika-Sotiriou, M., Kovacs, G., Larsson, H., de Carvalho, M. A. A. P., Rubiales, D., Russell, J., Dos Santos, T. M. M., & Vaz Pato, M. C. (2010). Cereal landraces for sustainable agriculture. A review. *Agronomy for Sustainable Development*, 30(2), 237–269. <https://doi.org/10.1051/agro/2009032>
21. Wang, J., Vanga, S., Saxena, R., Orsat, V., & Raghavan, V. (2018). Effect of Climate Change on the Yield of Cereal Crops: A Review. *Climate*, 6(2), 41. <https://doi.org/10.3390/cli6020041>
22. Bocci, R., Bussi, B., Petitti, M., Franciolini, R., Altavilla, V., Galluzzi, G., Di Luzio, P., Migliorini, P., Spagnolo, S., Floriddia, R., Rosi, G. L., Petacciato, M., Battezzato, V., Albino, A., Faggio, G., Arcostanzo, C., & Ceccarelli, S. (2020). Yield, yield stability and farmers' preferences of evolutionary populations of bread wheat: A dynamic solution to climate change. *European Journal of Agronomy*, 121, 126156. <https://doi.org/10.1016/j.eja.2020.126156>
23. R Core Team. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria, 2021; Available online: <https://www.R-project.org/> (accessed on 10 September 2022).
24. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis*; Springer: New York, NY, USA, 2016; ISBN 978-3-319-24277-4. Available online: <https://ggplot2.tidyverse.org> (accessed on 10 September 2022).
25. Le, S.; Josse, J.; Husson, F (2008). FactoMineR: An R Package for Multivariate Analysis. *J. Stat. Soft.*, 25, 1–18.
26. Kassambara, A.; Mundt, F (2020). Factoextra: Extract and Visualize the Results of Multivariate Data Analyses. R Package Version 1.0.7.2020. Available online: <https://CRAN.R-project.org/package=factoextra> (accessed on 10 June 2022)
27. Alqudah, A. M., & Schnurbusch, T. (2017). Heading Date Is Not Flowering Time in Spring Barley. *Frontiers in Plant Science*, 8, 896. <https://doi.org/10.3389/fpls.2017.00896>
28. Niu, Y., Chen, T., Zhao, C., & Zhou, M. (2021). Improving Crop Lodging Resistance by Adjusting Plant Height and Stem Strength. *Agronomy*, 11(12), 2421. <https://doi.org/10.3390/agronomy11122421>
29. Guarda, G., Padovan, S., & Delogu, G. (2004). Grain yield, nitrogen-use efficiency and baking quality of old and modern Italian bread-wheat cultivars grown at different nitrogen levels. *European Journal of Agronomy*, 21(2), 181–192. <https://doi.org/10.1016/j.eja.2003.08.001>
30. Adhikari, B., & Shrestha, J. (2018). Agronomic Performance and Correlation Study in Barley (*Hordeum vulgare* L) Genotypes. <https://doi.org/10.21276/sjavs.2018.5.12.6>

Capitolo 3

Preliminary characterization of the malting quality of malting barley and landraces of wheat and rye grown in a Mediterranean environment (southern Italy)

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Abstract

This study examined the quality characteristics of some barley, wheat and rye varieties before and after malting. The objective was to evaluate the malting ability of barleys derived from an agronomic test carried out in southern Italy, whose agronomic potential had been previously tested and found to be excellent in the test environment. Concurrently, the malting potential of some wheat and rye landraces traditionally cultivated in Calabria was evaluated for the first time, as a first step for future in-depth studies in terms of malting optimization on specific genotypes. Malting barleys presented ideal characteristics, with little variability identified for some characteristics. Wheat and rye landraces showed varied although promising results. Appropriate malting programs adapted to these particular raw materials must be implemented to optimize the malts obtained from them.

Keywords: barley; wheat; rye; landrace; malting.

1. Introduction

Malting is the process prior to brewing, necessary to trigger the germination of a grain and the biochemical and physiological events within it. This process allows to obtain a new raw material for the brewing industry, whose enzyme content will allow high molecular weight molecules to be broken down into simpler substances that can be used by yeast during fermentation (Briggs, 1998). The majority of studies on the subject concern barley, a prime material for the malting industry. Less is known about the malting of wheat and rye, especially with reference to old genotypes (Alfeo et al., 2018; Alfeo et al., 2021). Some studies have also been carried out on einkorn and emmer, in particular on the influence of glumes in malting and brewing, as well as their suitability as ingredients for the production of alternative beers that are increasingly appealing to consumers (Marconi et al., 2013; Mayer et al., 2011). New barley varieties are tested and released year by year (Asres et al., 2018; Craine et al., 2022). Field tests have been carried out on barley to investigate how growth conditions and genotype may influence certain phyco-chemical properties (Tomasi et al., 2019; Marconi et al., 2020). Italian malt production increased from 68,116 to 79,501 tons from 2008 to 2021, although 148,000 tons were imported from foreign countries in the same year (assobirra.it). The objective of this work was to evaluate the quality attributes of the malts obtained from cereal varieties grown in the Calabria region. The malting barley varieties used in this study are well-known for their suitability for the malting and brewing processes. Nonetheless, they exhibited excellent agronomic traits in the test environment based on the results of a previous investigation. To the best of our knowledge, no information are available on the malting quality of rye and wheat landraces under study, commonly used for other purposes, e.g., as raw material for bread production. In particular, limited information is available on the malting of rye compared to wheat and barley. In order to

gather more information on the malting of the three rye landraces under study, three different germination times were adopted to assess their impact in terms of malt quality.

2. Materials and methods

2.1. Raw materials

Cereals (malting barley, rye, wheat) harvested in the experimental field of *Azienda Regionale per lo Sviluppo dell'Agricoltura Calabrese* (ARSAC) located in San Marco Argentano (Calabria, South Italy) (39°38'N, 16°13'E, 250 m a.s.l.), in the season 2019-2020, were evaluated in terms of grain and malting quality parameters, thanks to the collaboration of the Italian Brewing Research Centre (CERB) in Perugia. The following varieties were subjected to malting: five malting barley varieties (Concerto, Fortuna, Laureate, Planet, Quench), two landraces of wheat (Mazzancoio, Rosia), three landraces of rye (Pollino, Sila, Aspromonte).

2.2. Chemical analysis

Analyses on grains, malted grains and worts were conducted according to the Analytica European Brewery Convention (EBC, 2007). Barley, wheat and rye grains have been subjected to a series of analyses prior to malting. In particular, the grain moisture (%) was determined by EBC method 3.2; the germination energy (GE, %) by EBC method 3.6.2; the water sensitivity (%) as difference between the germinative energy (GE, %) measured in the 4 mL and 8 mL tests; the thousand kernel weight (TKW, g) by EBC method 3.4; the total nitrogen (% d.m.) by EBC 3.3.1; the total proteins (% d.m.) as total nitrogen multiplied by 6.25 for barley and 5.7 for rye and wheat, respectively; the sieving test (%) by EBC method 3.11.1.

After the malting process, barley, wheat and rye malts have undergone a series of analyses. The moisture content (%) was measured by EBC method 4.2; total nitrogen (TN, % dm) by EBC method 4.3.1 and soluble nitrogen (SN, % d.m.) by EBC method 4.9; proteins and soluble proteins (% d.m.) were calculated as total nitrogen (TN, % d.m.) or soluble nitrogen (SN, % d.m.), respectively, multiplied by 6.25 for barley and by 5.7 for rye and wheat; the Kolbach Index (%) was calculated as soluble to total nitrogen ratio; wort color (EBC units) was determined by EBC method 4.7.1; friability (%) by EBC method 4.15; extract (% d.m.) was measured by EBC method 4.5.1; fermentability (%) using EBC method 4.11.1; pH by EBC method 4.5.1.; wort viscosity (mPa x s) was determined with by EBC method 4.8; β -glucan content (mg/L) of the Congress wort was determined using EBC method 8.13.1; free amino nitrogen content (FAN, mg/100 g d.m.) by EBC method 4.10.

2.3. Malting scheme

Malting was performed in triplicate according to the following schedule. The steeping process was conducted at the same temperature of 14 °C for a total of 28 h, reaching a steeping-out moisture of 45%. In particular, the steeping steps were as follows: 1st steeping of 5 h; 1st air rest of 12 h; 2nd steeping of 4 h; 2nd air rest of 5 h; 3rd steeping for 2 h. The germination step ended after 6 days at 14 °C for barley and wheat. In particular, rye grains were germinated using three different germination times (4, 5, 7 days) at 14 °C. Thereafter, germinating grains were dried and kilned for 17 h, as detailed below: 12 h at 55 °C, 2.5 h at 72 °C with recirculation at 20%, and 2.5 h at 82 °C with recirculation at 70%. Rootlets were removed from the malted samples at the end of the kilning process.

2.4. Statistical analysis

Grain analysis data before malting are reported as the average of three measurements plus expanded uncertainty (U). Data on malting for each species were subjected to the analysis of

variance (ANOVA) using Rstudio, R version 4.1.0 (R Core Team, 2021). Statistical significance was assessed using F test, while differences between means were tested by means of Tukey's Test HSD at $p \leq 0.05$ significance.

3. Results and discussion

3.1. Grain analysis

3.1.1. Barley

Table 1. Quality characteristics of barley.

Variable	Concerto	Fortuna	Laureate	Planet	Quench
Moisture (%)	13.2 ± 0.5	13.1 ± 0.5	13.2 ± 0.5	13.4 ± 0.5	13.1 ± 0.5
Total nitrogen (TN, % d.m.)	1.92 ± 0.09	1.67 ± 0.09	1.72 ± 0.09	1.75 ± 0.09	1.82 ± 0.09
Total proteins (TS, % d.m.)	12.0 ± 0.6	10.4 ± 0.6	10.8 ± 0.6	10.9 ± 0.6	11.4 ± 0.6
Thousand kernel weight (g)	42.3 ± 1.2	45.9 ± 1.2	41.9 ± 1.2	43.0 ± 1.2	38.2 ± 1.2
Germination energy (%)	100 ± 1	100 ± 1	100 ± 2	100 ± 1	100 ± 2
Water sensitivity (%)	2 ± 2	2 ± 2	0 ± 2	12 ± 4	25 ± 6.5
S. f. I > 2.8 mm (%)	77.5 ± 3.1	74.7 ± 3.4	83.3 ± 2.6	66.6 ± 4.1	46.6 ± 5.8
S. f. II 2.8-2.5 mm (%)	17.4 ± 2.5	18.8 ± 2.7	13.3 ± 2.2	23.8 ± 3.1	38.4 ± 4.4
S. f. I + II (> 2.5 mm) (%)	95.0 ± 1.8	93.5 ± 2.0	96.7 ± 1.5	90.3 ± 2.4	85.0 ± 3.0
S. f. III (2.5-2.2 mm) (%)	4.2 ± 1.2	5.4 ± 1.2	2.6 ± 1.2	7.4 ± 1.2	12.9 ± 1.2
S. f. IV (< 2.2 mm) (%)	0.8 ± 0.9	0.9 ± 0.9	0.5 ± 0.9	2.0 ± 0.9	1.9 ± 0.9
S. f. V (broken, extraneous, other) (%)	0.2 ± nd	0.2 ± nd	0.2 ± nd	0.3 ± nd	0.2 ± nd
S. f. V + VI (%)	0.9 ± nd	1.1 ± nd	0.7 ± nd	2.3 ± nd	2.0 ± nd

Values are expressed as means ± U (n = 3). S. f., sieving fraction; nd, not defined; d.m., dry matter.

Table 1 summarizes the quality characteristics of the barley varieties under study. For the correct storage of barley, it is necessary that the moisture content of the seed is low enough to prevent the development of undesirable organisms and, at the same time, ensure that germination-related enzymes activity is inactivated (Girma Tura et al., 2020). Considering all barley varieties, the average moisture content (13.2%) was in line with the value reported in the Analytica European Brewery Convention (<14.5%). The protein level is influenced by various elements, such as weather, soil conditions, and fertilization operations (Steiner et al., 2011). Standard values for a good malting barley range between 10% d.m. and 11% d.m. (Analytica-EBC, 2007). However, the influence of the growth environment may exert less of an effect on protein content than genetic factors (Luo et al., 2019). In this study, the protein content ranged from 10.4 to 12.0% d.m. for Fortuna and Concerto, respectively. Thus, with the exception of Concerto and Quench, the other varieties analysed showed a suitable protein content in line with standards. The thousand kernel weight was in line with standard values, ranging from 38.2 g to 45.9 g for Quench and Fortuna, respectively. Based on the specific thousand kernel weight value, the different varieties fell into different classes. In particular, Quench can be defined as light barley (reference: 37–40 g), Laureate, Concerto and Fortuna as medium weight barley (reference: 41–44 g), while Fortuna as heavy barley (reference: >45 g). A homogeneous and ready germination is necessary to obtain quality malt. During the post-harvest period, the germination rate naturally tends to increase (Frančáková et al., 2012). Germination energy was above the minimum reference value (>95%) resulting the same (100%) for all the varieties, while the water sensitivity was in the range 0%–25% for Laureate and Quench, respectively; overall, Concerto, Fortuna and Laureate were characterized by a very low water sensitivity (<10%), while Planet and Quench varieties exhibited a higher water sensitivity (11–25%, respectively). All barley varieties showed good values for the plump barley fraction (I + II), Quench being the variety with the lowest value (85.0%). Notably, Concerto and Laureate can be referred as premium malting barley (≥95%), while Fortuna and Planet can be considered as fine malting barley (>90 %) in relation to the sieving test results.

3.1.2. Wheat grains analyses

Table 2. Wheat landraces quality parameters.

Variable	Mazzancoio	Rosia
Moisture (%)	13.6 ± 0.5	13.9 ± 0.5
Total nitrogen (TN, % d.m.)	2.31 ± 0.09	2.79 ± 0.09
Total proteins (TP, % d.m.)	13.1 ± 0.6	15.9 ± 0.6
Thousand kernel weight (g)	36.3 ± 1.2	44.4 ± 1.2
Germination energy (%)	94 ± 5	93 ± 6
Water sensitivity (%)	5 ± 4	3 ± 5.5
S. f. I >2.8 mm (%)	95.9 ± 1.5	95.5 ± 1.5
S. f. II 2.8-2.5mm (%)	3.2 ± 1.3	3.5 ± 1.3
S. f. I + II (>2.5 mm) (%)	99.1 ± 1.2	99.0 ± 1.2
S. f. III (2.5-2.2 mm) (%)	0.4 ± 1.2	0.5 ± 1.2
S. f. IV (<2.2 mm) (%)	0.5 ± 0.9	0.5 ± 0.9
S. f. V (broken, extraneous, other) (%)	0.0 ± nd	0.0 ± nd
S. f. V + VI (%)	0.5 ± nd	0.5 ± nd

Values are expressed as means ± U (n = 3). S. f., sieving fraction; nd, not defined; d.m., dry matter.

Table 2 summarizes wheat quality characteristics. The average moisture content of wheat (13.7%) was higher than that of barley and rye (13.2 and 13.6%, respectively), and below the reference threshold value for dry cereals (14.5%). A different protein content characterized the wheat landraces under study. Proteins with high-molecular weight can be found in wheat compared to barley, which play an important role in terms of improving foam characteristics and haze formation and stability in wheat beers (Faltermaier et al., 2014); at the same time, it should be remembered that an inversely proportional relationship between protein content and extract exists (Hübner et al., 2010). Total protein ranged between 13.1 and 15.9% d.m. for Mazzancoio and Rosia, respectively. There was also variability in the thousand kernel weight values among the old wheat populations (36.3 g for Mazzancoio, 44.4 g for Rosia), in line with the findings reported in Alfeo et al. (Alfeo et al., 2021) regarding wheat landraces. Germination energy was slightly lower than the reference value for both landraces (94 and 93% for Mazzancoio and Rosia, respectively), which also exhibited a low water sensitivity (5% for Mazzancoio and 3% for Rosia). The sieving test results showed that about 99% of the seeds fell into the fraction I+II (>2.5 mm), overall higher value than even barley (mean value equals to 92.1%).

3.1.3. Rye grains analyses

Table 3. Rye landraces quality parameters.

Variable	Aspromonte	Pollino	Sila
Moisture (%)	13.4 ± 0.5	13.8 ± 0.5	13.5 ± 0.5
Total nitrogen (TN, % d.m.)	2.27 ± 0.09	2.20 ± 0.09	2.25 ± 0.09
Total proteins (% dm)	13.0 ± 0.6	12.6 ± 0.6	12.9 ± 0.6
Thousand kernel weight (g)	28.9 ± 1.2	28.9 ± 1.2	23.5 ± 1.2
Germination energy (%)	97 ± 4	99 ± 3	97 ± 4
Water sensitivity (%)	5 ± 5	2 ± 3.5	0 ± 4
S. f. I >2.8 mm (%)	11.9 ± 8.9	9.8 ± 9.1	3.6 ± 9.6
S. f. II 2.8-2.5mm (%)	39.0 ± 4.4	41.7 ± 4.7	23.4 ± 3.1
S. f. I + II (>2.5 mm) (%)	50.9 ± 7.5	51.5 ± 7.4	27.0 ± 10.6

S. f. III (2.5-2.2 mm) (%)	34.1 ± 1.2	37.7 ± 1.2	42.0 ± 1.2
S. f. IV (<2.2 mm) (%)	14.9 ± 0.9	10.6 ± 0.9	30.7 ± 0.9
S. f. V (broken, extraneous, other) (%)	0.2 ± nd	0.0 ± nd	0.0 ± nd
S. f. V + VI (%)	15.1 ± nd	10.6 ± nd	30.7 ± nd

Values are expressed as means ± U (n = 3). S. f., sieving fraction; nd, not defined; d.m., dry matter.

Results on rye quality characteristics are shown in Table 3. The average moisture content of rye (13.6%) resulted higher than that of barley (13.2%) and similar to that of wheat (13.7%), therefore below the reference threshold value for dry cereals (14.5%). The protein content (12.8% d.m., on average) was higher than that of barley but lower than that of wheat. However, protein values for all landraces, if compared with the recommended values for wheat malt, were within the acceptable limits (11–13%). Thousand kernel weight resulted the lowest above all cereals, with a mean value of 27.1 g, in line with the results reported in another study on malting of rye (Wang et al., 2018). In particular, Aspromonte and Pollino presented the same value (28.9 g) and Sila the lowest (23.5 g). For an optimal and homogeneous malting process is important to use as plump and similarly sized kernels as possible, preferably held within the 2.5 mm sieve. This variability, especially in terms of seed morphology, is not surprising, as rye kernels of these populations generally show variability in shape and size, commonly appearing long and more or less tapered, since they are cross-pollinated plants. Germination energy resulted greater than 95%, while the water sensitivity resulted very low, ranging from 0 to 5% for the Sila and Aspromonte landraces, respectively. Referring to the percentage of seeds in the fractions I+II (>2.5 mm), Aspromonte and Pollino landraces exhibited the plumpest kernels (50.9% and 51.5%, respectively), in line with the results reported in Wang et al. (2018) with reference to conventional rye varieties, while Sila exhibited the tiniest grains (27.0%). When compared with the barley values for the same parameter, there is a marked discrepancy, as this quality trait is related to the size and shape of the seed.

3.2. Malt

3.2.1. Barley malt

Data on barley malts quality traits are reported in Figure 1. The moisture content ranged between 4.0 and 5.8% for Planet and Concerto, respectively. The moisture content of Quench and Concerto (5.7 and 5.8%, respectively) was slightly above the optimal range for pale malts (3–5%). There was no statistically difference between the moisture content of Fortuna and Laureate (4.4 and 4.6%). Friability values were ≥80%, except for Quench malt (75%). Planet, Laureate and Fortuna presented the highest friability among varieties with values above 95%. Referring to color, Planet resulted the darkest colored malt (6.4 EBC unit). Quench and Concerto showed the lowest values of 3.4 and 3.0 EBC unit, respectively, while intermediate values have been recorded for Fortuna and Laureate (4.5 and 5.3 EBC unit). The pH did not vary significantly between varieties, ranging from 5.80 to 6.12 for Planet and Quench, respectively. Results were in line with standard values (5.60–6.00), with the exception of Quench variety, that showed a value above the high threshold. The extract ranged between 80.3 and 82.5% d.m. for Planet and Concerto, respectively, in line with the standard values reported for barley malt (79–82% d.m. for pale malts). Fermentability ranged from 83 to 86% for Quench and Laureate variety, respectively, showing good values for this quality trait. There was a high variability regarding the β-glucan content, which differed between the barley varieties with values ranging from 69 to 226 mg/L for Fortuna and Quench variety, respectively. While Planet, Laureate and Fortuna presented the lowest values, it should be noted that remarkably high levels were measured for Concerto and Quench. Measured viscosity was in line with the standard values (<1.56 mPa × s) for all genotypes, for an average value of 1.46 mPa × s. Kolbach Index varied in the range 38–57% for

Quench and Laureate, respectively; all varieties showed a value above the recommended range (35–45 %), with the exception of Quench malt.

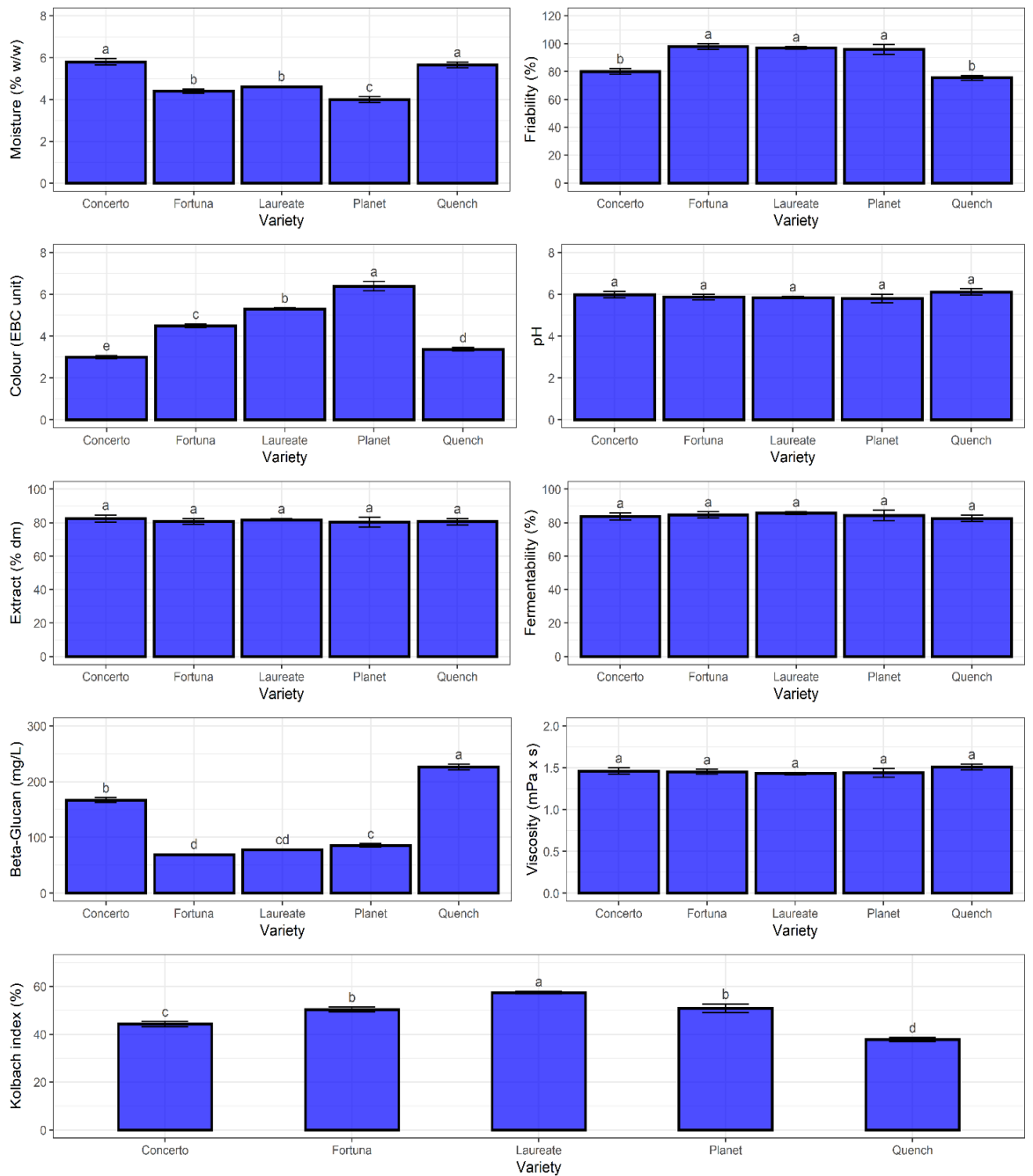


Figure 1. Quality characteristics of barley malts. Values are expressed as means (error bars = standard deviation). Different letters indicate a statistically significant difference ($p \leq 0.05$).

3.2.2. Wheat malt

The main quality traits of malted wheats are reported in Figure 2. The variability observed between the two landraces under study was such that no statistically significant difference was detected between the measured variables. As a result of the heat treatment performed during the kilning process, moisture content varied between 5.2 and 4.8% for Rosia and Mazzancoio, respectively. Friability ranged between 52 and 57% for Rosia and Mazzancoio varieties; these values differ from those reported in Alfeo et al. (2018; 2021). In particular, it should be noted that Mazzancoio wheat has a characteristic caryopsis with a predominantly vitreous fracture, therefore lower friability values would have been expected according to the above references. The color varied from 5.6 to 7.4 EBC unit for Mazzancoio and Rosia, respectively. The same kilning process was adopted for both cereals, hence the difference in color could be probably related to the nitrogen content of Rosia malt. The pH values were below the standard range (5.9–6.1), with observed values of 5.77 and 5.79 for Rosia and Mazzancoio, respectively. Mazzancoio malt showed a slightly higher extract (82.8% d.m.) than Rosia malt (80.6% d.m.), both just below the reference value (>83% d.m.) for standard wheat malt. Fermentability varied in the range 77 and 80% for Rosia and Mazzancoio, respectively. In particular, the value observed for Rosia was slightly below the minimum standard level of 78%. β -Glucan content resulted higher in Rosia (70 mg/L) than in Mazzancoio (64 mg/L), but lower if compared to barley. This is not surprising as these cell-wall components are represented in lower quantities in wheat than in barley or oats (Faltermaier et al., 2014). Viscosity was nearly the same for both varieties (1.75 mPa x s for Rosia and 1.74 mPa x s for Mazzancoio), therefore within the maximum standard value reported for wheat malt (<2.00 mPa x s). The KI was the same for both landraces and equals to 53%, higher than the results reported in Alfeo et al. (2021) and in relation to the standard values reported in Faltermaier et al. (2014).

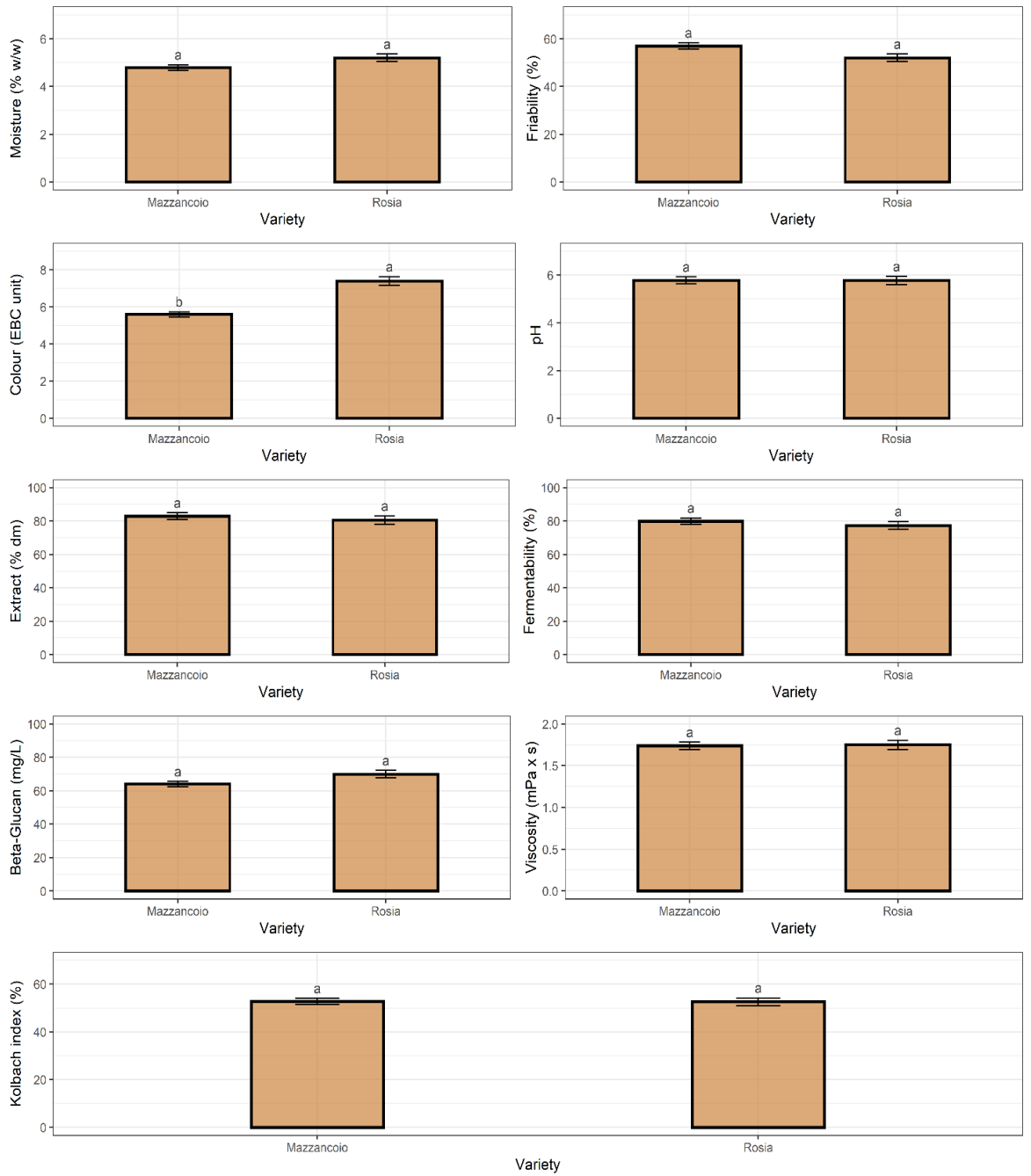


Figure 2. Quality characteristics of wheat malts. Values are expressed as means (error bars = standard deviation). Different letters indicate a statistically significant difference ($p \leq 0.05$).

3.2.3. Rye malt

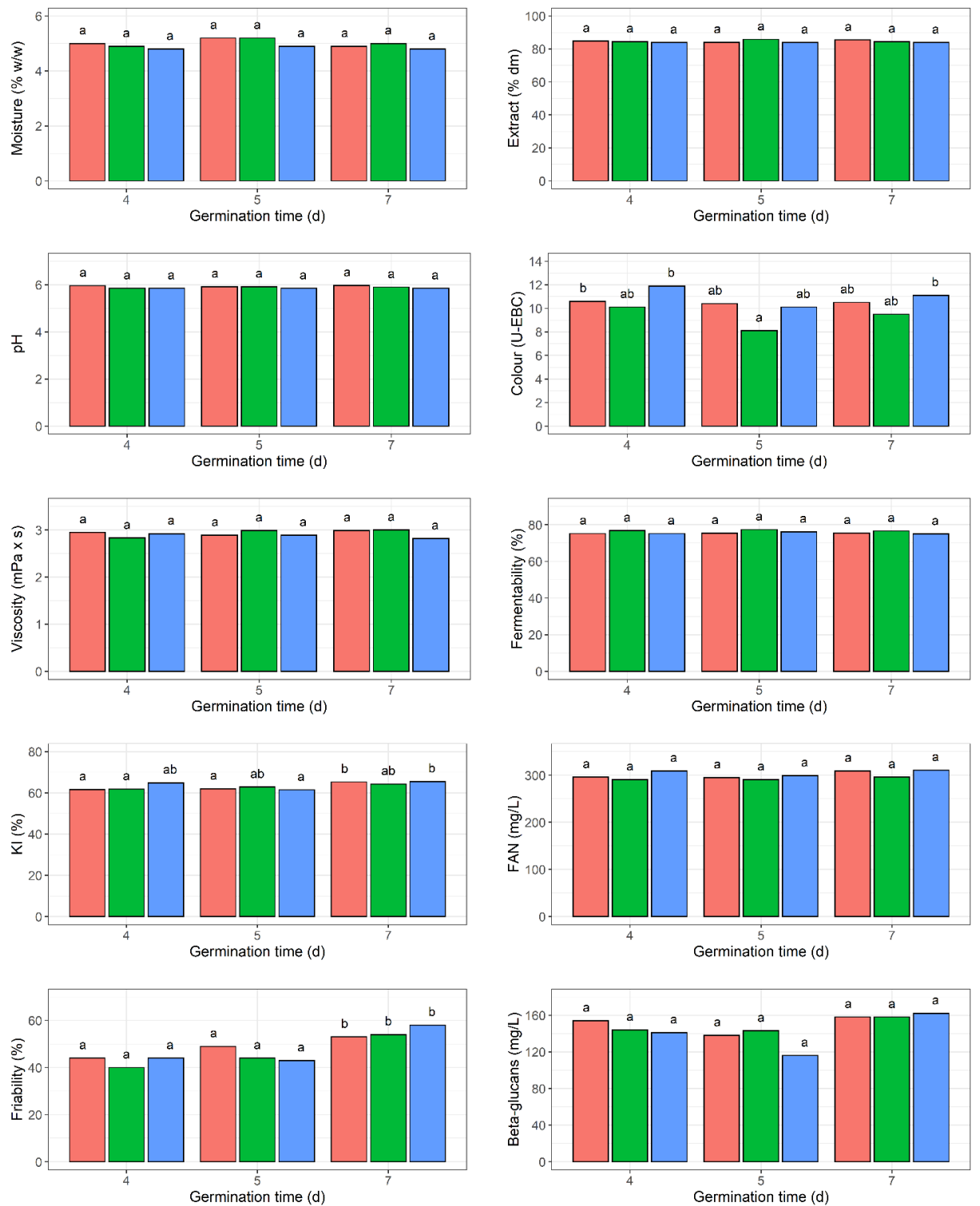


Figure 3. Quality characteristics of rye malts. Comparison between rye landraces using different germination times at the malting process. Values are expressed as means. Different letters indicate a statistically significant difference ($p \leq 0.05$). Different colors within the bars refer to different varieties (pink for Aspromonte, green for Pollino, blue for Sila).

Figure 3 summarizes the malting quality attributes of the three rye landraces. The average moisture content was 5.4%, 5.0%, and 4.9% for Aspromonte, Pollino, and Sila, respectively. The average extract resulted 80.5% for Aspromonte, 83.9% for Sila, 80.6% for Pollino. All landraces

showed a similar pH for a mean value of 5.95 for Aspromonte, 5.85 for Sila, 5.89 for Pollino landraces. The highest color values were found in the samples germinated for 4 days for all the landraces. Comparing the average color of the malts obtained from the three cereals under study, rye exhibited the darkest color (10.2 EBC unit, on average). The mean viscosity values were higher compared to those detected for barley and wheat in the Congress worts, and resulted as follows: 2.94 mPa x s for Aspromonte, 2.94 mPa x s for Pollino, 2.87 mPa x s for Sila, however without any significant difference. The fermentability was quite similar among the landraces with the highest average value of 76.9% for Pollino, while Sila and Aspromonte presented values of 75.5% and 75.4, respectively. The average protein content was high for all rye malts, ranging from 13.21 to 13.61% d.m. for Pollino and Sila landraces, respectively (data not shown). The FAN content varied in the range 293–306 mg/L for Pollino and Sila, respectively. The average malt friability were quite similar between the landraces ranging from 46% for Pollino to 48.6% for Aspromonte. As expected, significant differences were found after seven days of germination by reason of an increased malt modification. The average β -glucans level was high compared to the results for wheat malts, ranging from 140 to 150 mg/L for Sila and Aspromonte, respectively. The Kolbach index varied adopting different days of germination, the highest value being observed for all the landraces after seven days of germination (63%, on average).

4. Conclusions

The above results showed that barley presented optimal values for almost all quality attributes for both grains and malts, as expected. The main reason why these barley varieties, whose malting qualities are well known, were subjected to a qualitative evaluation was the intention to carry out a more specific assessment in relation to the test area in which they have been cultivated. It was therefore possible to conclude that these genotypes resulted well adapted to the reference test area, given the results of the field and technological studies. In particular, the malting barley varieties Fortuna, Laureate and Planet presented higher values for friability, color and a lower content of β -glucans. Hence, these three varieties ranked among the tested barleys in terms of malting traits. Wheat presented good germination energy and low water sensitivity, but higher protein content than barley; thousand kernel weight resulted variable between wheat local populations; concerning the sieving test, more than 95% of the kernels fell in the first fraction (> 2.8 mm). Wheat malts were characterized by low viscosity and low β -glucan content if compared with rye. The reported results are promising and show that such wheat populations exhibit more than adequate malting traits overall. Rye landraces showed good germination energy and low water sensitivity such as wheats, and higher protein content than barley; moreover, they were characterized by low values for the thousand kernel weight. Worts obtained by rye malts were characterized by greater viscosity and β -glucan content than those obtained by barley or wheat malts. Results showed that all the rye landraces have similar malting qualities, but Sila rye should be rejected for use in malting because of the small size of the kernels as well as for exhibiting an excessive beta glucan content even after prolonged germination. In conclusion, it would be interesting to modify the malting conditions changing other parameters during malting, e.g. germination temperature or degree of steeping, to assess their influence on the characteristics of wheat and rye malts, with the final aim to use them for the creation of a special beer based on these two traditional landraces. Therefore, as this represented a preliminary study, subsequent research will be necessary to optimize the malting process according to specific methods. In subsequent work inherent in malting trials, barley varieties showed qualities suitable for the malting industry. Therefore, laboratory tests also confirmed that quality malt can be obtained from barley grown in a Mediterranean environment.

References

1. Briggs, D. E., Boulton, C. A., Brookes, P. A., Stevens, R. (2004). *Brewing Science and practice*, Woodhead Publishing Limited and CRC Press, LLC.
2. Alfeo, V., Jaskula-Goiris, B., Venora, G., Schimmenti, E., Aerts, G., & Todaro, A. (2018). Screening of durum wheat landraces (*Triticum turgidum* subsp. *Durum*) for the malting suitability. *Journal of Cereal Science*, 83, 101–109. <https://doi.org/10.1016/j.jcs.2018.08.001>
3. Alfeo, V., De Francesco, G., Sileoni, V., Blangiforti, S., Palmeri, R., Aerts, G., Perretti, G., & Todaro, A. (2021). Physicochemical properties, sugar profile, and non-starch polysaccharides characterization of old wheat malt landraces. *Journal of Food Composition and Analysis*, 102, 103997. <https://doi.org/10.1016/j.jfca.2021.103997>
4. Marconi, O., Mayer, H., Chiacchieroni, F., Ricci, E., Perretti, G., Fantozzi P. "The influence of glumes on malting and brewing of hulled wheats" *Journal of the American Society of Brewing Chemists*, 71(1), (2013) 41-48.
5. Mayer, H., Marconi, O., Perretti, G., Sensidoni, M., Fantozzi, P. "Investigation on suitability of hulled wheats for malting and brewing" *Journal of the American Society of Brewing Chemists* 69, 2, (2011) 116-120.
6. Asres, T., Tadesse, D., Wossen, T., & Sintayehu, A. (2018). Performance Evaluation of Malt Barley: From Malting Quality and Breeding Perspective. *Journal of Crop Science and Biotechnology*, 21(5), 451–457. <https://doi.org/10.1007/s12892-018-0199-0>
7. Craine, E. B., Bramwell, S., Ross, C. F., & Murphy, K. M. (2022). From Ground to Glass: Evaluation of Unique Barley Varieties for Craft Malting Craft Brewing, and Consumer Sensory. *Beverages*, 8(2), 30. <https://doi.org/10.3390/beverages8020030>
8. Tomasi, I., Sileoni, V., Marconi, O., Bonciarelli, U., Guiducci, M., Maranghi, S., Perretti, G. "Effect of Growth Conditions and Genotype on Barley Yield and β -Glucan Content of Kernels and Malt." *Journal of Agricultural and Food Chemistry*, 2019, 67 (22), 6324–6335.
9. Marconi, O., Tomasi, I., Sileoni, V., Bonciarelli, U., Guiducci, M., Maranghi, S., Perretti, G. "Effects of Growth Conditions and Cultivar on the Content and Physicochemical Properties of Arabinoxylan in Barley." *Journal of Agricultural and Food Chemistry*, 2020, 68 (4), 1064–1070.
10. AssoBirra, available at https://www.assobirra.it/wp-content/uploads/2022/09/AnnualReport_2022_14_web.pdf (Accessed on 11/10/2022)
11. European Brewery Convention, 2007. *Analytica-ebc*, fifth ed. Fachverlag Hans Carl, Nurnberg, Germany.
12. Girma, A., Abera, S., Dereje, B., & Chalchisa, T. (2020). Effects of Germination Temperature and Time on Malt Quality of Temash Barley (*Hordeum vulgare* L.). *Journal of Food Science and Nutrition*, 8, 63–73. <https://doi.org/10.11648/j.jfns.20200803.13>
13. Luo, H., Harasymow, S., Paynter, B., MacLeod, A., Izydorczyk, M. S., O'Donovan, J. T., & Li, C. (2019). Genetic and environmental impact on protein profiles in barley and malt: Genetic and environmental impact on protein profiles in barley and malt. *Journal of the Institute of Brewing*, 125(1), 28–38. <https://doi.org/10.1002/jib.532>
14. Frančáková, H., Líšková, M., Bojňanská, T., & Mareček, J. (2012). Germination index as an indicator of malting potential. *Czech Journal of Food Sciences*, 30(4), 377–384. <https://doi.org/10.17221/314/2010-CJFS>
15. Steiner, E., Gastl, M., & Becker, T. (2011). Protein changes during malting and brewing with focus on haze and foam formation: A review. *European Food Research and Technology*, 232(2), 191–204. <https://doi.org/10.1007/s00217-010-1412-6>
16. Faltermaier, A., Waters, D., Becker, T., Arendt, E., & Gastl, M. (2014). Common wheat (*Triticum aestivum* L.) and its use as a brewing cereal - a review: Common wheat and its use as a brewing cereal. *Journal of the Institute of Brewing*, 120(1), 1–15. <https://doi.org/10.1002/jib.107>
17. Hübner, F., & Arendt, E. K. (2010). Comparison of Protein Degradation as a Consequence of Germination Time and Temperature in Rye and Barley Malts. *Journal of the American Society of Brewing Chemists*, 68(4), 195–203. <https://doi.org/10.1094/ASBCJ-2010-0923-01>
18. Wang, Y., Jin, Z., Barr, J., Gillespie, J., Simsek, S., Horsley, R., & Schwarz, P. (2018). Micro-Malting for the Quality Evaluation of Rye (*Secale cereale*) Genotypes. *Fermentation*, 4(3), 50. <https://doi.org/10.3390/fermentation4030050>

Capitolo 4

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Malting process optimization of an Italian common wheat landrace (*Triticum aestivum* L.) through response surface methodology and desirability approach

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Abstract

Local and alternative raw materials are of growing interest to the malting and brewing industry. These include wheat landraces, old varieties characterized by high protein content and resistance to biotic and abiotic stress. One of the basic ingredients of beer is malt, i.e., a grain that have been subjected to germination under controlled conditions. Malting awakens the seed's physiological activity, thereby triggering its chemical and structural modification. The response surface methodology (RSM) was used to assess the impact of three independent variables (germination time, germination temperature, degree of steeping) on certain traits defining malt quality. Germination time and temperature exerted a major role during the malting experiments. A desirability function was applied to predict the best combination of parameters that would optimize the desired outcomes. After 6 days, at 18 °C and 42 g/100 g, the following results were achieved: extract 81.6% d.m., Kolbach index (KI) 38.3%, free amino nitrogen (FAN) 116 mg/100 g, apparent attenuation limit (AAL) 82.9%. The response surface methodology (RSM) proved to be largely suitable for the optimization of the malting process under study. Its implementation through the R statistics programming language and environment provided an alternative and valuable resource for conducting the statistical analysis and optimization.

Keywords: common wheat; landrace; micro-malting; response surface methodology; desirability function.

Abbreviations: RSM, response surface methodology ; KI, Kolbach index; FAN, free amino nitrogen; AAL, apparent attenuation limit; d.m., dry matter; MEBAK, Mitteleuropäische Brau- und Analysenkommision; FO, first order; TWI, two-way interaction; PQ, pure quadratic; L, lowest acceptable value; T, desired target; U, highest acceptable value.

1. Introduction

The rise of human civilization over the centuries has been closely related to the cultivation of cereals. Their domestication started in the Fertile Crescent from wild progenitors and old hulled grains such as einkorn, emmer and spelt (Arzani & Ashraf, 2017). Wheat (*Triticum* spp.) is one of the most extensively cultivated crop and a staple food for humans and livestock, thanks to its environmental adaptation and nutritional value (Curtis, Rajaram, & Gómez Macpherson, 2002). Not solely destined for breadmaking, it has also been used in malting and brewing since ancient

times, alongside barley, which dominates the market. Historical evidence, traceable to the alchemist Zosimus of Panopolis, reports its use as an ingredient in a fermented beverage in ancient Egypt (Meussdoerffer, 2009). The high protein content and the presence of high molecular weight substances, which affect wort viscosity, require some technical considerations regarding its use in beer brewing (Faltermaier, Waters, Becker, Arendt, & Gastl, 2014). Furthermore, the malting of wheat involves some adjustments to the process to avoid damaging to the sprouting grains, due to the absence of husks.

Research has shown the suitability of some durum wheat landraces (*Triticum turgidum* subsp. *durum* Desf.) for malting (Alfeo et al., 2018, 2021), although common wheat (*Triticum aestivum* L.) has traditionally been preferred (Briggs, 1998). The term landrace refers to genotypes characterised by yield stability under low-input, resistance to adverse environmental conditions (Zeven, 1998) and to various fungal diseases, that ordinarily hamper wheat plant growth and production (Wang et al., 2021, 2022; Xu et al., 2018). Despite steady but lower yields, greater plant height and protein content if compared to modern improved cultivars (Mefleh et al., 2019), local farmers maintain and exchange these local varieties, thereby limiting genetic erosion (Jaradat, 2013). In a study comparing different wheat genotypes (landraces, primitive, old, spelt varieties), landraces were found to possess the highest total carotenoid content (Hussain, Larsson, Kuktaite, Olsson, & Johansson, 2015). In addition, a greater amount of minerals such as iron, zinc and magnesium has been detected compared to wheat cultivars (Akcura & Kokten, 2017). The use of locally grown and processed raw materials could represent an alternative to imports, an opportunity to reduce costs and environmental impact. Moreover, it would create and consolidate a strong bond with the territory for local farms and breweries in terms of quality and marketing (De Simone et al., 2021).

The research for the most suitable and efficient process conditions is an ongoing challenge in food and beverage manufacturing (Malekjani & Jafari, 2020). The optimization of a product with desired attributes results in the solution of a multivariate problem, as numerous traits mutually contribute to define its final quality (Myers, Montgomery, & Anderson-Cook, 2009). A statistical and mathematical technique, widely used in the agro-industrial field for this purpose, is the response surface methodology (RSM) (Manuel Pais-Chanfrau, Núñez-Pérez, del Carmen Espin-Valladares, Vinicio Lara-Fiallos, & Enrique Trujillo-Toledo, 2021), which combines the creation and analysis of experimental designs with optimization procedures (Breig & Luti, 2021). This methodology has also been widely adopted for the malting optimization of cereals and pseudocereals (Djameh et al., 2015; Muñoz-Insa, Gastl, Zarnkow, & Becker, 2011; Phiarais, Schehl, Oliveira, & Arendt, 2006; Zarnkow et al., 2007).

In this study, RSM was applied for the optimization of the malting of a common wheat landrace of Italian origin. Several factors affecting malt quality are involved and examined during small-scale malting: steeping times and temperatures, the effect of aeration, the application of growth regulating additives or agents with anti-microbial activity, and so on (Briggs, 1998). In particular, the influence of diverse combinations of three parameters, i.e., germination time, germination temperature, and degree of steeping, was evaluated on four malt quality traits. The variables evaluated were: extract, Kolbach index (KI), apparent attenuation limit (AAL), free amino nitrogen (FAN). The extract is represented by the dry substances of the malt (Back, 2008). KI is defined by the ratio of soluble to total nitrogen (Briggs, 1998). Increasing the days of germination from 4 to 7 days determines greater KI (Faltermaier, Waters, Becker, Arendt, & Gastl, 2013). Consequently, the activity of proteolytic and amylolytic enzymes may be differently influenced in wheat malt. Indeed, the breakdown of gliadin and complex polysaccharides is enhanced when the KI is in the range 37.6–42.7% (Jin, Du, Zhang, & Guo, 2014). FAN corresponds to the quantity of low-molecular-weight substances, mainly represented by amino acids, necessary for the correct metabolism and development of yeast during fermentation (Briggs, Boulton, Brookes, & Stevens, 2004). Most of these substances, that are formed during grain germination (Burger & Schroeder, 1976), serve as precursors of chemical compounds defining beer flavour (Ferreira & Guido, 2018). AAL represents that part of the extract that can be

metabolized by yeasts during fermentation. The higher the presence of assimilable sugars, the greater the percentage of fermentability should be (Narziß & Back, 2012). Following the analysis of response surfaces, the next stage was to search for the optimal combination of malting parameters to increase the extract and the AAL, while keeping the KI and the FAN within the lower and upper limits recommended for wheat malt. Hence, a multi-objective optimization was conducted using a desirability function in order to predict the best combination of germination time, germination temperature and degree of steeping optimizing the selected malt quality traits.

2. Materials and methods

2.1. Raw material

A common wheat landrace (*Triticum aestivum* L.), locally known as Rosia, was used in the micro-malting experiments. Grain was harvested and supplied by a local farm in the province of Vibo Valentia, in Calabria region (southern Italy). Moisture and protein content of the raw material were 12.1 g/100 g and 13.7 g/100 g (d.m.), respectively. It is well known that physiological status, seed variety and storage conditions affect seed dormancy, with high temperatures increasing the propensity to germinate (Briggs, 1998). The initial germinative energy was 89% and the grains were incubated at a constant temperature of 40 °C for a week, until a final germinative energy of 95% was reached.

2.2. Statistical analysis

Response surface methodology and desirability approach were used for the design, the analysis and the optimization of the malting process of wheat using R statistics programming language and environment (R Core Team, 2021), software version 4.1.0 (2021-05-18). The R packages used for data analysis and graphical representation included: ‘rsm’ (Lenth, 2009); ‘performance’ (Lüdecke et al., 2021); ‘MASS’ (Venables & Ripley, 2002); ‘desirability’ (Kuhn, 2016); ‘ggplot2’ (Wickham, 2016); ‘ggthemes’ (Arnold, 2021); ‘patchwork’ (Pedersen, 2020); ‘metan’ (Olivoto and Lúcio, 2020). A brief description of the above-mentioned packages can be found in Table S1. A randomized 23 factorial design was applied to study the effect of three malting parameters, germination time (d), germination temperature (°C) and degree of steeping (g/100 g), on some quality traits of malted wheat. Six axial runs at $\alpha = \pm 1$ and four centre points were combined to the base design to allow the estimation of pure experimental error (Borkowski, 2008) and to fit second order model responses, obtaining a face-centred composite design, with 18 runs overall. The region for such a design can be represented in a three dimensional space in the shape of a cube, where the areas of interest and operability are identical (Myers et al., 2009). Preliminary experiments were carried out in advance to select the boundaries of the experimental region. The defined ranges for the independent variables, with their coded and natural values, are reported in Table 1.

Table 1

Experimental factors with coded and actual levels.

Factor	Unit	Symbol	Level		
			-1	0	+1
Germination time	d	A	4	5	6
Germination temperature	°C	B	12	15	18
Degree of steeping	g/100 g	C	40	43	46

The malt quality traits analysed were: extract, Kolbach index (KI), free amino nitrogen (FAN), apparent attenuation limit (AAL). Other equally important malt-related traits were measured (Table S2), but the data were not further analysed since their predictive models were not suitable for additional investigations (significant lack of fit). A multivariate polynomial regression

analysis was carried out to describe the trend of each response upon variations of the independent variables. To fit quadratic models and obtain regression and ANOVA outcomes, the following rsm function (extension of the lm function (Lenth, 2009)) was implemented:

$$rsm(formula=Y \sim FO(xA,xB,xC) + TWI(xA,xB,xC) + PQ(xA,xB,xC), data) \quad (1)$$

where Y is the dependent variable; FO, TWI and PQ are the linear, two-way interaction and pure quadratic functions, respectively; xA, xB, xC are the independent variables expressed in coded units; data is the matrix containing all combinations of experimental settings and observed values. A correlation analysis, based on Pearson correlation method, was also performed to measure the linear dependence between each pair of variables. The graphical representation of the correlation analysis is illustrated in Fig. S1. All the malting parameters combinations for each run and observed values for each response are reported in Table 2. For an effective and intuitive comparison of the measured attributes upon each individual experimental run, a min-max normalisation was applied to the original values so that they can be expressed in the identical scale. Therefore, they were associated to new values ranging from 0 to 1, for the lowest and the highest recorded response, respectively, including decimals within the two limits.

Table 2

Levels of the malting parameters, in coded and natural units, and malt quality traits.

run.order	std.order	xA	xB	xC	A	B	C	Extract (% d.m.)	KI (%)	FAN (mg/100 g)	ALL (%)
1	6	1	-1	1	6	12	46	81.2	33.7	108	78.4
2	2	1	-1	-1	6	12	40	81.4	32.5	102	79.1
3	8	1	1	1	6	18	46	81.3	43.5	136	82.3
4	4	1	1	-1	6	18	40	81.5	36.8	110	81.2
5	10	0	0	0	5	15	43	81.6	34.8	119	80.8
6	3	-1	1	-1	4	18	40	81.1	31.9	102	80.9
7	7	-1	1	1	4	18	46	81.2	37.0	119	81.1
8	17	0	0	-1	5	15	40	81.4	33.7	105	77.4
9	11	0	0	0	5	15	43	81.2	36.0	120	78.2
10	16	0	1	0	5	18	43	81.4	36.3	115	81.1
11	5	-1	-1	1	4	12	46	79.1	23.9	69	74.4
12	15	0	-1	0	5	12	43	80.7	28.7	87	77.3
13	14	1	0	0	6	15	43	82.2	38.6	124	82.2
14	18	0	0	1	5	15	46	81.3	37.7	126	80.8
15	12	0	0	0	5	15	43	82.0	35.6	113	80.5
16	13	-1	0	0	4	15	43	80.2	29.4	90	77.1
17	1	-1	-1	-1	4	12	40	77.9	22.3	63	75.0
18	9	0	0	0	5	15	43	81.5	35.4	112	80.2

A: germination time (d); B: germination temperature (°C); C: degree of steeping (g/100 g); d.m.: dry matter; KI: Kolbach index; FAN: free amino nitrogen; AAL: apparent attenuation limit.

2.3. Micro-malting

Each run involved malting 1 kg of wheat that was previously cleaned and sieved in order to remove broken and foreign kernels. Experiments were conducted in the micro-malting plant of the Research Centre Weihenstephan for Brewing and Food Quality (Freising, Germany), Technical University of Munich (TUM). A standard steeping regime (R- 110.00.008, 4.) (Methner, 2018) was performed during 48 h at different temperatures, according to the experimental schedule. In particular, all samples were initially steeped for 5 h, followed by 19 h of air rest; on

the second day, a second steeping of 4 h was followed by 20 h of air rest. The final degree of steeping was adjusted for each sample on the first day of germination. It was also checked daily in order to keep it at the target value, by spraying the required amount of water on grains. Germination was conducted varying all the malting parameters according to the experimental design (Table 2). Samples were subjected to automatic rotation to prevent compacting of the mass and to allow its aeration. A standard kilning method was applied as follows: 16 h at 50 °C; 1 h at 60 °C; 1 h at 70 °C; 5 h at 80 °C. After kilning, each malt sample was left to cool and rootlets were removed. Subsequently, samples were stored for a week to allow uniform redistribution of the residual moisture until analyses.

2.4. Malt and wort analyses

Malts and corresponding worts were analysed in duplicate ($n = 2$) and the mean of each measurement has been reported (Table 2). Analyses were carried out at the accredited laboratory of the Research Centre Weihenstephan for Brewing and Food Quality, Technical University of Munich, according to the Mitteleuropäische Brau- und Analysenkommision (MEBAK) (Methner, 2018) methods, using Congress and isothermal 65 °C mash programs. The moisture content of grains and malts was determined by R-110.40.020 and R-200.18.020 methods, respectively, as the difference in mass prior to and after drying at defined temperature. The germination energy was assessed by R-110.29.612 (AUBRY Method). The extract content was determined on the Congress mash by R-205.01.080 (EBC-Method). The nitrogen content (crude protein) of grains and malts was determined by R-110.41.030 and R-200.20.030, respectively, according to Kjeldahl Method. The protein percentage was obtained using the conversion factor 5.7. Soluble nitrogen was measured according to R-205.11.030 according to Kjeldahl method. Kolbach index was calculated as ratio of soluble to total nitrogen by R-205.12.999 (EBC-Method). Free amino nitrogen (FAN) was determined by R-205.14.111 (EBC-Method). Apparent attenuation limit (AAL) was quantified according to R-205.16.080 (Fermentation Tube Method).

3. Results and discussion

3.1. Model fitting and evaluation

Fig. 1 illustrates the comparison between the four responses analysed for each experimental run.

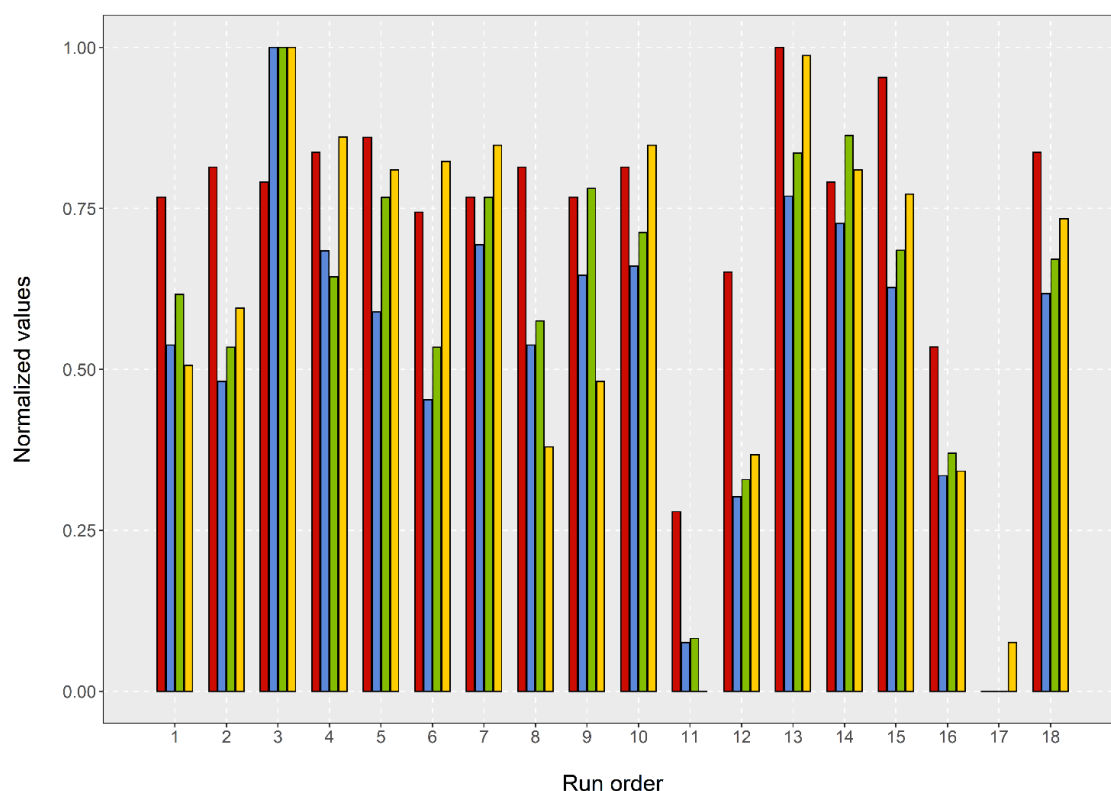


Fig. 1. Bar chart of normalized responses for each experimental run. Each bar is filled with a specific colour as indicated below: extract (red); KI: Kolbach index (blue); FAN: free amino nitrogen (green); AAL: apparent attenuation limit (yellow). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The maximum extract (82.2% d.m.) was reached at experimental run No. 13 (6 days, 15 °C and 43 g/100 g). All the other selected variables (KI, FAN, AAL) presented the greatest values at experimental run No. 3, i.e., at the highest values for each malting parameter (6 days, 18 °C and 46 g/100 g). On the contrary, minimum extract, KI and FAN were obtained at experimental run No. 17 (4 days, 12 °C and 40 g/100 g), while the lowest value for AAL (74.4%) was attained at the experimental run No. 11 (4 days, 12 °C and 46 g/100 g). Overall, longer germination times allowed all dependent variables to be maximised, albeit at different temperatures and degrees of steeping. This can be attributed to a gradual progression of the inner modification of the sprouting grain, with the consequent breakdown of macromolecules into substances of lower-molecular-weight supporting the seedling growth (Rani & Bhardwaj, 2021). Indeed, seed imbibition triggers the germination process with the subsequent activation or neoformation of hydrolytic enzymes with different and specific activity (Ali & Elozeiri, 2017).

Table 3 shows the least squares estimates of the coded coefficients, computed according to function (1), with the associated statistical parameters for extract, KI, FAN and AAL.

Table 3
Output of the regression analysis for the selected responses.

Response	Statistics	Intercept	xA	xB	xC	xA:xB	xA:xC	xB:xC	xA ²	xB ²	xC ²
Extract	Estimate	81.549	0.810	0.620	0.080	-0.638	-0.213	-0.138	-0.323	-0.473	-0.173
	Sdt. Error	0.124	0.100	0.100	0.100	0.112	0.112	0.112	0.192	0.192	0.192
	t value	655.232	8.095	6.196	0.800	-5.700	-1.900	-1.298	-1.679	-2.458	-0.891
	Pr(> t)	<0.001	<0.001	<0.001	0.4470	<0.001	0.0940	0.2539	0.1317	0.0394	0.3953
	Sign.	***	***	***		***				*	
KI	Estimate	35.182	4.060	4.440	1.860	-1.075	0.150	1.125	-0.917	-2.417	0.782
	Sdt. Error	0.271	0.218	0.218	0.218	0.244	0.244	0.244	0.419	0.419	0.419
	t value	129.788	18.632	20.376	8.536	-4.413	0.616	4.618	-2.190	-5.773	1.871
	Pr(> t)	<0.001	<0.001	<0.001	<0.001	0.0023	0.5552	0.0017	0.0599	0.0004	0.0982
	Sign.	***	***	***	***	**		**		***	

FAN	Estimate	114.167	13.700	15.300	7.600	-6.625	1.125	3.875	-5.333	-11.333	3.167
	Sdt. Error	1.691	1.360	1.360	1.360	1.520	1.520	1.520	2.612	2.612	2.612
	t value	67.496	10.076	11.253	5.590	-4.358	0.740	2.549	-2.042	-4.340	1.212
	Pr(> t)	<0.001	<0.001	<0.001	<0.001	0.0024	0.4804	0.0342	0.0755	0.0025	0.2600
	Sign.	***	***	***	***	**		*	.	**	
AAL	Estimate	79.333	1.470	2.240	0.340	NA	NA	NA	NA	NA	NA
	Sdt. Error	0.288	0.386	0.386	0.386	NA	NA	NA	NA	NA	NA
	t value	275.746	3.803	5.803	0.880	NA	NA	NA	NA	NA	NA
	Pr(> t)	<0.001	0.0019	<0.001	0.3933	NA	NA	NA	NA	NA	NA
	Sign.	***	**	***		NA	NA	NA	NA	NA	NA

KI: Kolbach index; FAN: free amino nitrogen; AAL: apparent attenuation limit. Sign., significance codes: '***' 0.001, '**' 0.01, '*' 0.05, '.' 0.1, ' ' 1; NA, Not Applicable.

The comparison and the selection between candidate models for each dependent variable was based upon: model performance metrics such as RMSE (Root Mean Squared Error), Sigma or RSE (Residual Standard Error), AIC (Akaike's Information Criteria), BIC (Bayesian Information Criteria); the significance of each regression terms (p-value <0.05); the values of R² and adjusted R². The coefficient of determination R² gives an indication of the amount of variance explained by a statistical model (Nakagawa, Johnson, & Schielzeth, 2017). It is influenced by the presence of many predictors and high-order polynomials, with the potential consequence of overfitting and decrease in the predictive ability of a regression model. Conversely, the adjusted R² is computed considering the number of regression coefficients, allowing a more reliable estimation of the analysed responses (Minitab Blog Editor, 2013). A marked discrepancy between them indicates the presence of non-significant terms within a model (Myers et al., 2009). Therefore, the models with the less difference between the two coefficients of determination and the greater adjusted R² were considered.

Regression coefficients with a p-value greater than 0.05 were discarded. Selected models presented adjusted R² values equal or higher than 0.90, with the sole exception of AAL. Details on comparison within each model for each response are reported in the supplementary material (Table S3).

Replicated runs at the centre of the experimental region enabled to check model adequacy by means of the lack-of-fit test. A significant outcome (p-value <0.05) indicates an unreliable model in adequately representing a response of interest (Anderson & Whitcomb, 2016). All models presented a non-significant lack of fit, therefore they were selected for further data analysis (Table 4).

Table 4

Statistical parameters used to test the goodness of the fitted models.

	Extract	KI	FAN	AAL
Model	Quadratic	Quadratic	Quadratic	Linear
F-value	19.14	104.5	36.4	16.32
p-value	<0.001	<0.001	<0.001	<0.001
R²	0.9556	0.9916	0.9762	0.7776
Adjusted R²	0.9057	0.9821	0.9493	0.73
Lack of fit (p-value)	0.5866	0.2469	0.4771	0.5325

KI: Kolbach index; FAN: free amino nitrogen; AAL: apparent attenuation limit.

Moreover, the models adequacy was assessed by the analysis of the externally studentized residuals, as well as through the comparison of the predicted and actual responses. Compared to the residuals obtained directly from the regression analysis, the studentized ones, i.e. the difference between observed and predicted values (Petzoldt, 2017) scaled with constant variance (Myers et al., 2009), are more appropriate for revealing possible outliers. The combination of the externally studentized residuals against the predicted response values showed a random distribution (Fig. S2), suggesting that the variance of the original observations was constant

(Myers et al., 2009) for all values of each malting quality attribute. Fig. S3 shows the distribution of residuals by the experimental run order. All points depicted in each subplots for all responses presented a random scatter. No points lay above or below the straight red lines, which means no outliers could be detected. Fig. S4 shows the distribution of points derived from the combination of predicted versus experimental responses. A good model prediction is expected when points lie near or above the straight line, otherwise under- or over-prediction is represented by points located below or beyond it, respectively (Breig & Luti, 2021). In each subplots, points lay close to the line, indicating that there is little deviation between the aforementioned and observed points. This is also evidenced by the high values of the adjusted R^2 , with the exception of subplot d, where a lower predictive power was evident for AAL, whose adjusted R^2 was 0.73.

The graphical representation of each fitted surface is shown by means of contour and surface plots. In both cases, the degree of steeping was set at fixed levels to ensure optimal visualisation. Each line or curve illustrated in the graphs represents constant values of predicted response (Anderson & Whitcomb, 2016).

3.2. Extract

Extracts below 80% d.m. were measured at 4 days, 12 °C, at the minimum and maximum levels of degree of steeping (Table 2). The highest observed value (82.2% d.m.) was obtained at 6 days, 15 °C and 43 g/100 g. The limits of acceptability for this quality trait are differently defined for barley (>81% d.m.) and wheat (>83% d.m.) for beer brewing (Titze et al., 2013). Therefore, based on the observed results, no extract above the minimum reference value could be obtained for any combination of parameters. In another study concerning the malting of durum wheat landraces with malt protein content of less than 11%, extract values between 70.2% and 85.9% were detected (Alfeo et al., 2018). Our results could be related to the high starting protein content (13.7 g/100 g d.m.) of the raw material. As a matter of fact, a strong significant and negative correlation ($r = -0.75$) between extract and protein content has been detected (Fig. S1), as also reported in (Jin, Zhang, & Du, 2008) for common wheat malt and in (Fujita, Simsek, & Schwarz, 2020) regarding the malting of ancient wheat species. Since the correlation was negative, it is expected that the lower the protein degradation, the lower the amount of extract. Indeed, reduced extract values were obtained in conjunction with high protein percentages at 4 days of germination and at 12 and 15 °C, namely for poorly modified malts.

The response model with regression coefficients and coded variables is shown in Equation (2):

$$\text{Extract} = 81.549 + 0.81 \times xA + 0.62 \times xB + 0.08 \times xC - 0.638 \times xAxB - 0.2123 \times xAxC - 0.138 \times xBxC - 0.323 \times xA^2 - 0.473 \times xB^2 - 0.173 \times xC^2 \quad (2)$$

The ANOVA confirmed the quadratic model fit, which turned out to be significant (F-value = 19.14). The high adjusted R-squared (0.9057) and the non-significant lack of fit justified the high predictive ability of the selected quadratic model. Germination time, temperature and their interaction were highly significant (p-value <0.001); therefore, an increase in the predicted response is expected for higher levels of these independent variables, with the former exerting a greater effect when considered individually. However, their negative interaction term (Table 3) implies that the effect of time upon the response depends on the temperature levels, and vice-versa. Fig. 2 shows how the response changes depending on germination time and temperature when the degree of steeping is held constant. A stationary point of maximum response could be identified.

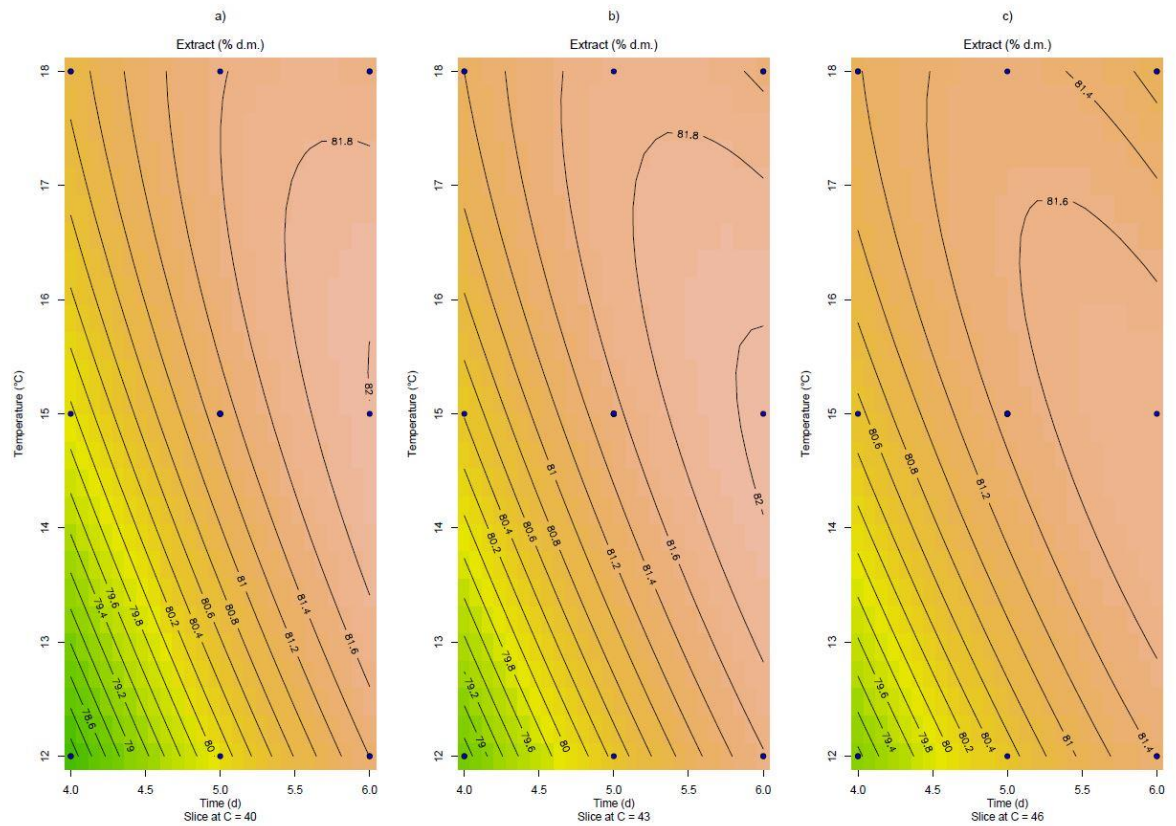


Fig. 2. Contour plots of the predicted extract for different germination times and temperatures. The degree of steeping C is held at a different fixed level in each subplot: a) C at 40 g/100 g; b) C at 43 g/100 g; c) C at 46 g/100 g.

Approximately the same extract (81.4% d.m.) was predicted after 6 days of germination at 12 °C, for a moisture content of 40 or 46 g/100 g, while a slightly higher value (81.6% d.m.) could be identified at 43 g/100 g. Moreover, from 4 days onwards, there was an increase in the response for gradually higher temperatures, at a steady moisture level. After 5 days, the extract changed considerably depending on the temperature adopted. Moving from the medium to the highest level of germination time, there was a clear drop in the predicted extract at 18 °C and with degrees of steeping varying from 43 g/100 g to 46 g/100 g. Actually, the greater the development of the seed at increased temperatures, the higher the loss of low molecular weight substances, which are destined for the development of acrospire and rootlets during germination (Belcar, Sekutowski, Zardzewiały, & Gorzelany, 2021). The latter are subsequently removed after kilning, with consequently malting losses (Fig. S5). The effect of the degree of steeping was almost negligible and not significant, in contrast to the results reported in (Muñoz-Insa, Selciano, Zarnkow, Becker, & Gastl, 2013) regarding the malting optimization of spelt. Actually, despite being frequently considered as related species, a divergence in the genetic sequence between wheat and spelt has been identified (Liu et al., 2018). In conclusion, the highest extract (82.0% d.m.) was predicted at 6 days, 15 °C and 43 g/100 g (Fig. 3), in accordance to the same experimental settings, thus confirming the good predictive capability of the selected model. A higher value could conceivably be obtained after 6 days under the conditions just mentioned, therefore outside the range of the experimental region. However, maximum limits for temperature and degree of steeping should be ruled out. In fact, prolonging germination under such conditions could lead to the spread of mould with deleterious consequences on malt quality and safety (Wolf-Hall, 2007).

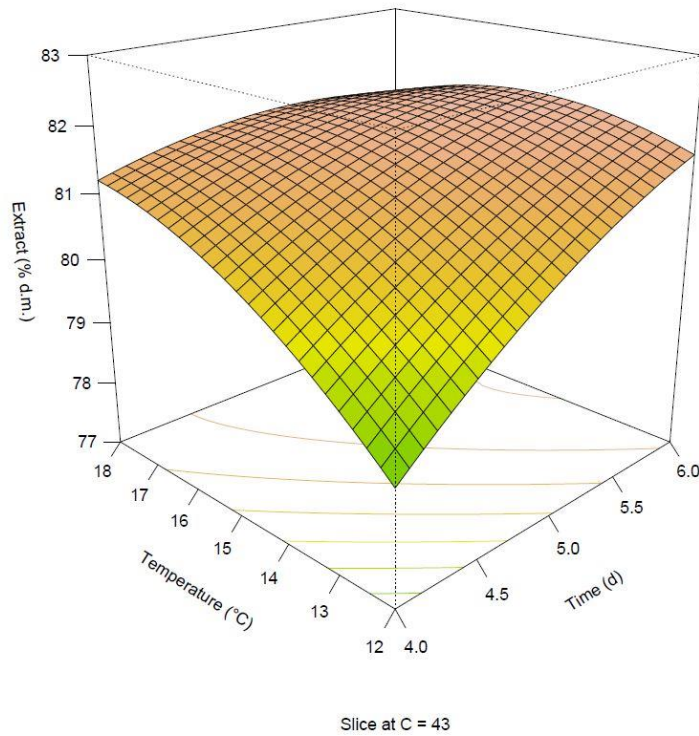


Fig. 3. Influence of germination time and temperature on the predicted extract. The degree of steeping C is held at 43 g/100 g.

3.3. Kolbach index

Different KI values were observed under completely different malting conditions. A germination period of 4 days at 12 °C and 40 g/100 g resulted in a minimum KI of 22.3%, while malting over 6 days at a temperature of 18 °C and 46 g/100 g led to a maximum KI value of 43.5% (Table 2). Recommended values for wheat malts are in the range 37–40 % (Narziß, 2005). There were only three observed values within the reference range, for the following combinations of parameters: 37.0% at 4 days, 18 °C, 46 g/100 g; 37.7% at 5 days, 15 °C, 46 g/100 g; 38.6% at 6 days, 15 °C, 43 g/100 g. It is interesting to note that a KI value of 37% was already achieved after only 4 days of germination, at the highest level of temperature and degree of steeping.

A quadratic model (3) described the response:

$$KI = 35.182 + 4.06 \times xA + 4.44 \times xB + 1.86 \times xC - 1.075 \times xAxB + 0.15 \times xAxC + 1.125 \times xBxC - 0.917 \times xA^2 - 2.417 \times xB^2 + 0.782 \times xC^2 \quad (3)$$

The adjusted R² was high and close to the unit (0.982) while the lack of fit was not significant. All the linear terms resulted highly significant, as well the interaction xAxB, xBxC, and the quadratic term for xB (Table 3). Therefore, all malting parameters exhibited a strong influence on this proteolytic specification. For each unit increase in the independent variables, larger values of KI were identified by the selected quadratic model. The predicted response varied according to the chosen degree of steeping. The extreme values (24–43%) were predicted at the highest humidity level. KI was positively correlated with extract (r = 0.84) and soluble nitrogen (r = 0.9), but negatively with protein content (r = - 0.95), in agreement with (Alfeo et al., 2018). The highest KI value (43.2%) was predicted after 6 days, 18 °C and 46 g/100 g (Fig. 4).

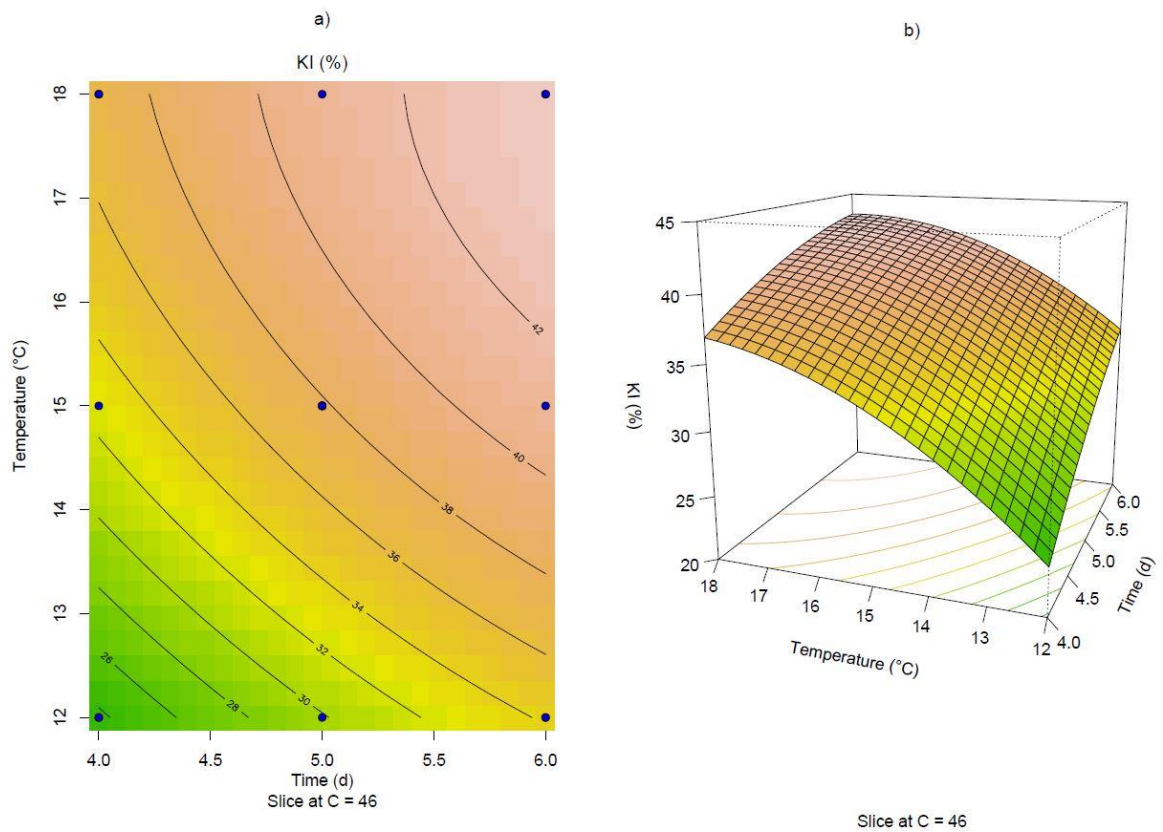


Fig. 4. Contour (a) and surface plot (b) for the influence of germination time and temperature on the Kolbach index (KI). The degree of steeping C is set as 46 g/ 100 g.

3.4. Free amino nitrogen

FAN content varied from 63 to 136 mg/100 g after 6 days, at 18 °C and 46 g/100 g. The recommended FAN values for wheat malt fall in the range of 90–120 mg/100 g, differently from what is suggested for barley malt, for which higher values (120–160 mg/100 g) are preferable (Narziß, 2005). Values below 90 mg/100 g were observed at 12 °C for different levels of germination time and degree of steeping. FAN contents above the maximum reference limit were obtained at 5 and 6 days for medium to high levels of temperature and degree of steeping (Table 2).

The FAN response was described by a quadratic model (4):

$$FAN = 114.167 + 13.7 \times xA + 15.3 \times xB + 7.6 \times xC - 6.625 \times xAxB + 1.125 \times xAxC + 3.875 \times xBxC - 5.333 \times xA^2 - 11.333 \times xB^2 + 3.167 \times xC^2 \quad (4)$$

The adjusted R² resulted higher than 0.90 and the lack of fit was not significant. xA, xB, xC resulted highly significant (Table 3). The interaction terms xAxB and xBxC were also significant, as well as the quadratic term for xB. Also in this case, as with KI, the response was strongly influenced by all process parameters. Comparing the response curves in each subplot in Fig. 5, a clear discrepancy can be seen: for increasing values of moisture content, at the same time and germination temperature, the predicted FAN content tended to be higher, particularly after 5 days. Values of more than 135 mg/100 g were predicted at temperatures of around 16–17 °C, for germination times of nearly 6 days (Fig. 5).

The maximum FAN content (136 mg/100 g) was predicted at 6 days, 18 °C and 46 g/100 g, in agreement with the observed value obtained in the malting experiment for the same combination of parameters, thus confirming the goodness of the selected model.

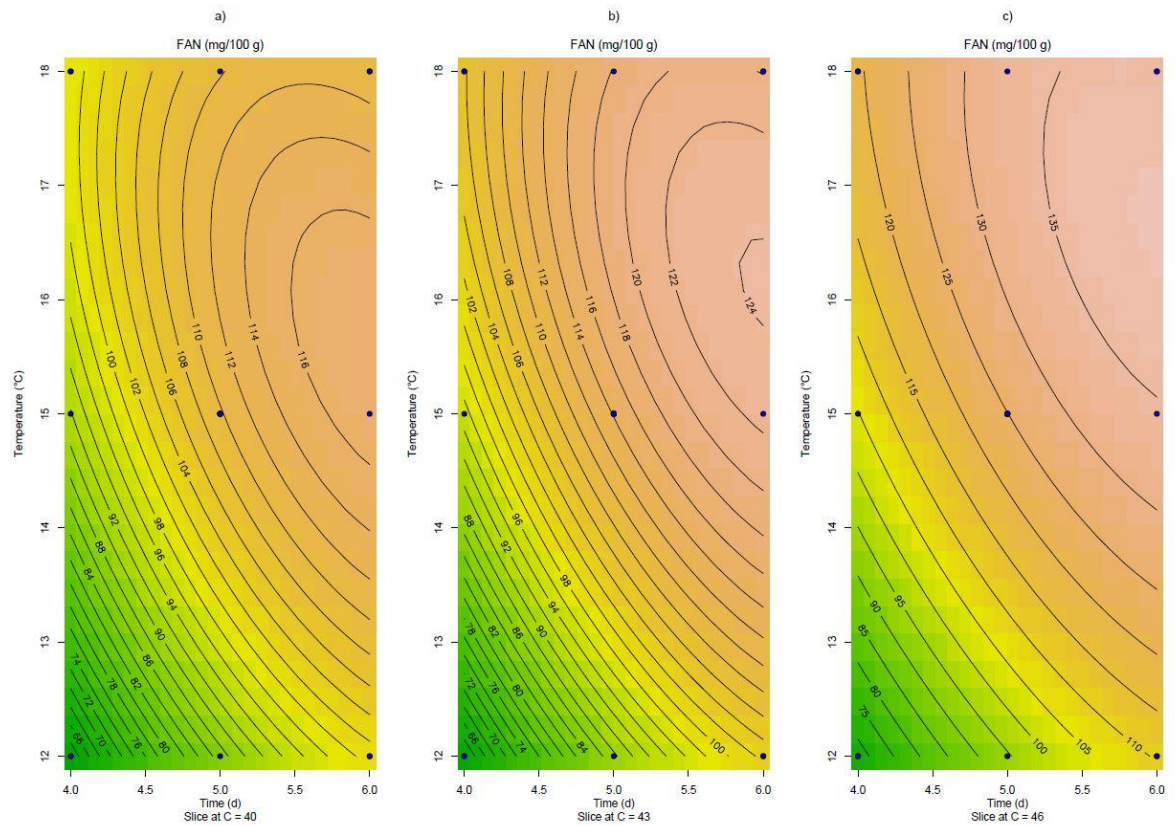


Fig. 5. Contour plots for the free amino nitrogen (FAN) against germination time and temperature. The degree of steeping *C* is kept fixed at a constant level in each subplot: a) *C* at 40 g/100 g; b) *C* at 43 g/100 g; c) *C* at 46 g/100 g.

3.5. Apparent attenuation limit

The observed AAL varied from 74.4% to 82.3% moving from 4 to 6 days, respectively, in both cases at the highest degree of steeping but for different temperatures (12 and 18 °C, respectively) (Table 2). AAL values above 79.0% are preferable for wheat malt (Narziß, 2005). Overall, malting at 12 °C did not result in malts with high AAL values. In the majority of the experiments carried out, it was possible to obtain values above the minimum threshold indicated as a reference (Table 2). The interaction and quadratic coefficients were not significant (*p*-value >0.05), so they were not considered within the model. Consequently, the selected linear model was significant (*F*-value = 16.32), with an adjusted *R*² of 0.73 and a not significant lack-of-fit (*p*-value >0.05).

AAL response was described by the following linear equation (5):

$$AAL = 79.333 + 1.47 \times xA + 2.24 \times xB + 0.34 \times xC \quad (5)$$

Germination time and temperature resulted significant, with the latter factor exerting a predominant effect on the response. As reported in Fig. 6, due to the absence of interaction and quadratic terms in the model, all straight lines appear parallel, thus describing an increase in the AAL percentage for higher levels of each malting parameter.

The degree of steeping exerted a lesser and even not significant impact. Since this is a linear model, it was not possible to identify an optimum condition for this response. The highest AAL (83.4%) was predicted at 6 days, 18 °C and 46 g/100 g (Fig. 6). A strong positive and significant correlation (*r* = 0.81) linked the response with the extract, although it is not necessarily the case that a higher extract corresponds to higher fermentability, as not all sugars in the wort can be assimilated by the yeast. A positive correlation was also found in relation to KI (*r* = 0.89); in particular, FAN content (*r* = 0.89) also exhibited a strong significant correlation, as also reported

by (Huerta-Zurita, Barr, Horsley, & Schwarz, 2020) in reference to barley malt, but in contrast to what was reported by (Krstanović, Mastanjević, Nedović, & Mastanjević, 2019) with reference to wheat malt.

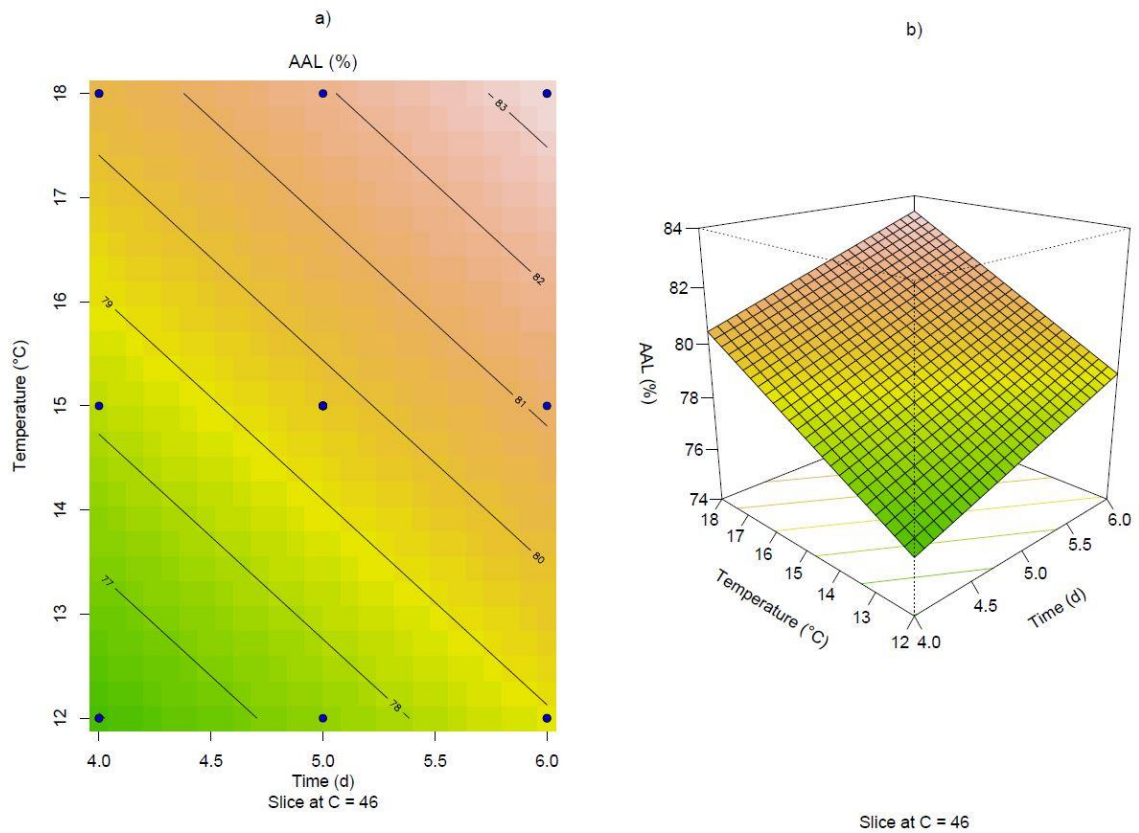


Fig. 6. Contour (a) and surface plot (b) for the apparent attenuation limit (AAL). The degree of steeping C is held at 46 g/100 g.

3.6. Multiple-response optimization

The desirability function approach proposed by Derringer and Suich (Derringer & Suich, 1980) was applied to find the best combination of malting parameters that simultaneously optimize the selected responses. This method allows each estimated response to be converted into a new individual desirability unit d_i , with $0 \leq d_i \leq 1$. A 'larger-is-better' function was chosen to maximise the extract and AAL, and a 'target-is-best' function to keep KI and FAN within a precise range. The optimization criteria for each response with their respective acceptance limits are reported in Table 5.

Table 5
Multi-response optimization criteria.

Response	Desirability function	Optimization boundaries
Extract	Larger-is-better	L = 80, U = 82
KI	Target-is-best	L = 37, T = 38.5, U = 40
FAN	Target-is-best	L = 90, T = 105, U = 120
AAL	Larger-is-better	L = 79, U = 82

L: lowest acceptable value; T: desired target; U: highest acceptable value.

A matrix of 78,141 entries containing combinations of experimental factors and each individual desirability was then created. The selection of the optimal values for each response was based on the standard acceptability criteria for wheat malt, with the sole exception of the

extract, for which values above 83% d.m. are considered acceptable for malting wheat (Faltermaier et al., 2014; Titze et al., 2013). Setting a minimum value of 83% d.m. would not have provided any useful solution, as the individual desirability calculated for the extract would in that case be equal to 0, thus nullifying the total desirability D , as shown below (6). For this reason, a value of 82.0% d.m. was chosen as maximum within the limits of the experimental design scores. Then, each individual function d_i , referring to a specified response, was combined to obtain a single overall desirability value D , ranging from 0 to 1, according to formula (6):

$$D = (d_{extract} \times d_{KI} \times d_{FAN} \times d_{AAL})^{1/4} \quad (6)$$

where the exponent is the reciprocal of the number of attributes examined. The aim was to find the best combination of individual desirability values maximising the overall desirability D . At the highest level of the time factor (6 days), D tended to increase for temperatures above 16 °C and for degrees of steeping between approximately 40 and 43 g/100 g (Fig. 7). The red region illustrated in Fig. 7 defines the area and the boundaries where the highest values of D could be found.

The optimal combination for the response variables was predicted at 6 days of germination, 18 °C and a degree of steeping of 42 g/100 g, with an overall D equals to 0.658 (Table 6). Slightly higher desirability values were actually calculated by the software, but they were not considered because they corresponded to temperatures and degrees of steeping expressed in decimals, such as not to be set in practice during malting.

Table 7

Predicted responses, individual and overall desirability for the optimized combination.

	Extract	KI	FAN	AAL
Predicted value	81.6% d.m.	38.3%	116 mg/100 g	82.9%
Individual desirability	0.808	0.878	0.265	1
Overall D	0.658			

d.m.: dry matter; KI: Kolbach index; FAN: free amino nitrogen; AAL: apparent attenuation limit.

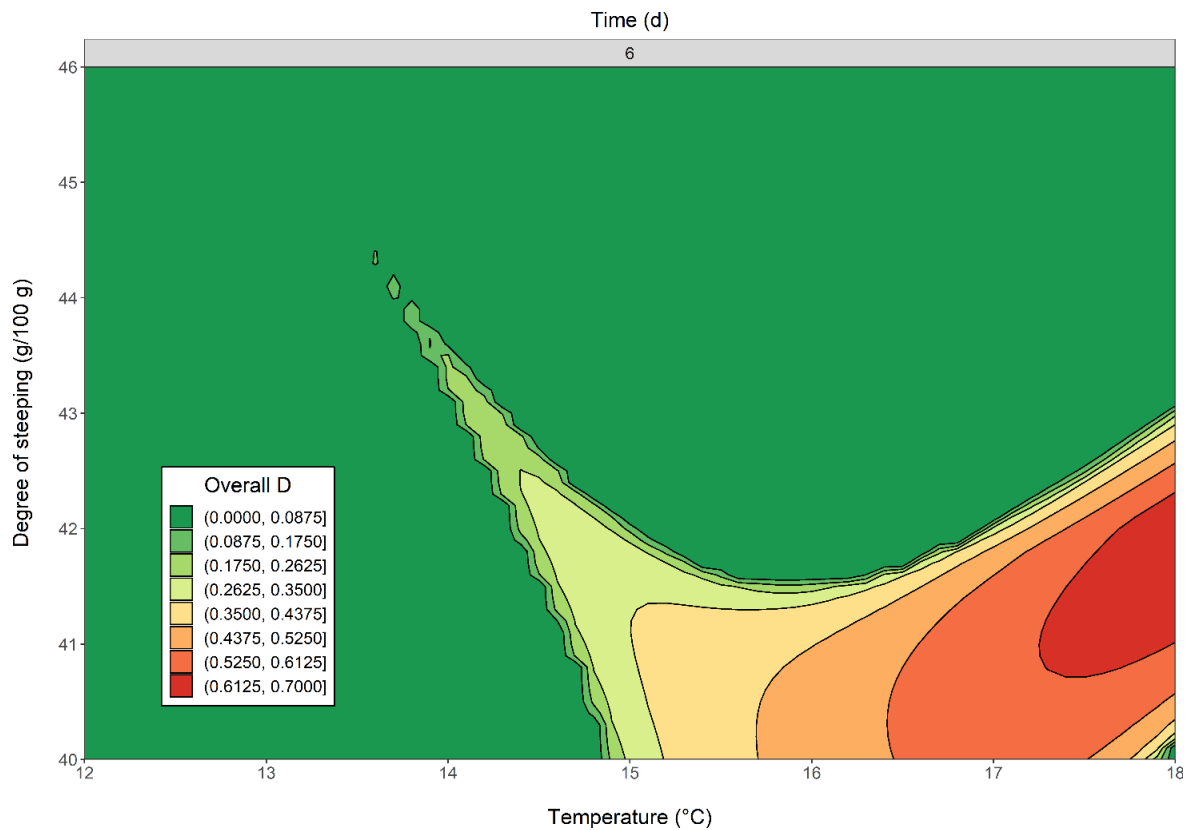


Fig. 7. Contour plot of the overall desirability D. The germination time is held at its optimum level (6 days) and it is represented in the upper part of the graph. Different colours refer to various ranges of overall desirability, as specified in the legend: from dark green to red colour there is an increase of the overall D. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Conclusion

In this study, the response surface methodology (RSM) was applied for the optimization of the malting process of a common wheat landrace. The R programming language proved to be versatile and appropriate for conducting the creation and the analysis of the experiments. The applied methodology provided insights into how the chosen malting parameters may influence the modification of the grain under study through the analysis of response surfaces. Germination time and temperature exerted a significant influence on all the examined responses. Proteolytic specifications (KI and FAN) were also significantly influenced by the degree of steeping. The optimization technique allowed to estimate the best combination of the independent variables at 6 days, 18 °C and 42 g/100 g, in order to maximise extract and AAL, while keeping KI and FAN within the desired ranges. This preliminary study is obviously limited to the boundaries of the region of this particular experimental design. Different levels and combinations of process parameters could, in fact, lead to different outcomes. Nevertheless, this study provided insight into how malting parameters affect the quality of malt obtained from a common wheat landrace. Finally, it would be interesting to brew a wheat beer with the malt thus optimized and evaluate it in terms of chemical and sensory evaluation. Its use as ingredient for beer brewing could contribute to a greater development of the local identity for breweries nearby the cultivation and maintenance areas of this cereal.

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CRedit authorship contribution statement

Antonio Calvi: Methodology, Investigation, Software, Formal analysis, Data curation, Writing – original draft, Visualization, Writing – review & editing. **Giovanni Preiti:** Conceptualization, Data curation, Writing – original draft. **Martina Gastl:** Resources, Supervision, Project administration, Validation. **Marco Poiana:** Resources, Supervision, Project administration, Validation. **Martin Zarnkow:** Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Antonio Calvi reports financial support was provided by Calabria Region.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

- Akcura, M., & Kokten, K. (2017). Variations in grain mineral concentrations of Turkish wheat landraces germplasm. *Quality Assurance and Safety of Crops & Foods*, 9, 153–159. <https://doi.org/10.3920/QAS2016.0886>
- Alfeo, V., De Francesco, G., Sileoni, V., Blangiforti, S., Palmeri, R., Aerts, G., et al. (2021). Physicochemical properties, sugar profile, and non-starch polysaccharides characterization of old wheat malt landraces. *Journal of Food Composition and Analysis*, 102, Article 103997. <https://doi.org/10.1016/j.jfca.2021.103997>

- Alfeo, V., Jaskula-Goiris, B., Venora, G., Schimmenti, E., Aerts, G., & Todaro, A. (2018). Screening of durum wheat landraces (*Triticum turgidum* subsp. *durum*) for the malting suitability. *Journal of Cereal Science*, 83, 101–109. <https://doi.org/10.1016/j.jcs.2018.08.001>
- Ali, A. S., & Elozeiri, A. A. (2017). Metabolic processes during seed germination. In J. C. Jimenez-Lopez (Ed.), *Advances in seed biology*. InTech. <https://doi.org/10.5772/intechopen.70653>.
- Anderson, M. J., & Whitcomb, P. J. (2016). *RSM simplified: Optimizing processes using response surface methods for design of experiments* (2nd ed.). Boca Raton: Taylor & Francis Group.
- Arnold, J. B. (2021). *ggthemes: Extra themes, scales and geoms for 'ggplot2'*. R package version 4.2.4 <https://CRAN.R-project.org/package=ggthemes>.
- Arzani, A., & Ashraf, M. (2017). Cultivated ancient wheats (*Triticum* spp.): A potential source of health-beneficial food products: Ancient wheats for healthy foods. *Comprehensive Reviews In Food Science And Food Safety*, 16, 477–488. <https://doi.org/10.1111/1541-4337.12262>
- Back, W. (2008). *Ausgewählte Kapitel der Brautechnologie*. Nürnberg: Hans Carl.
- Belcar, J., Sekutowski, T. R., Zardzewiały, M., & Gorzelany, J. (2021). Effect of malting process duration on malting losses and quality of wheat malts. *Acta Universitatis Cibiniensis. Series E: Food Technology*, 25, 221–232. <https://doi.org/10.2478/auaft-2021-0020>
- Borkowski, J. J. (2008). Center points. In F. Ruggeri, R. S. Kenett, & F. W. Faltin (Eds.), *Encyclopedia of statistics in quality and reliability*. John Wiley & Sons, Ltd. <https://doi.org/10.1002/9780470061572.eqr030>.
- Breig, S. J. M., & Luti, K. J. K. (2021). Response surface methodology: A review on its applications and challenges in microbial cultures. *Materials Today Proceedings*, 42, 2277–2284. <https://doi.org/10.1016/j.matpr.2020.12.316>
- Briggs, D. E. (1998). *Malts and malting* (1st ed.). Blackie Academic.
- Briggs, D. E., Boulton, C. A., Brookes, P. A., & Stevens, R. (2004). *Brewing science and practice*. Woodhead Publishing Limited and CRC Press, LLC.
- Burger, W. C., & Schroeder, R. L. (1976). Factors contributing to wort nitrogen. I. Contributions of malting and mashing, and effect of malting time. *Journal of the American Society of Brewing Chemists*, 34, 133–137. <https://doi.org/10.1080/03610470.1976.12006211>
- Curtis, B. C., Rajaram, S., & Gómez Macpherson, H. (2002). *Wheat for bread and other foods in Bread wheat, Improvement and production*. FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS Rome. Retrieved from <https://www.fao.org/3/y4011e/y4011e0w.htm#bm32>. (Accessed 22 June 2022).
- De Simone, N., Russo, P., Tufariello, M., Fragasso, M., Solimando, M., Capozzi, V., et al. (2021). Autochthonous biological resources for the production of regional craft beers: Exploring possible contributions of cereals, hops, microbes, and other ingredients. *Foods*, 10, 1831. <https://doi.org/10.3390/foods10081831>
- Derringer, G., & Suich, R. (1980). Simultaneous optimization of several response variables. *Journal of Quality Technology*, 12, 214–219. <https://doi.org/10.1080/00224065.1980.11980968>
- Djameh, C., Saalia, F. K., Sinayobye, E., Budu, A., Essilfie, G., Mensah-Brown, H., et al. (2015). Optimization of the sorghum malting process for pito production in Ghana: Sorghum malting process for pito production. *Journal of the Institute of Brewing*, 121, 106–112. <https://doi.org/10.1002/jib.191>
- Editor, M. B. (2013). Multiple regression analysis: Use adjusted R-squared and predicted R-squared to include the correct number of variables. Retrieved from <https://blog.minitab.com/en/adventures-in-statistics-2/multiple-regression-analysis-use-adjusted-r-squared-and-predicted-r-squared-to-include-the-correct-number-of-variables>. (Accessed 30 May 2022) Accessed.
- Faltermaier, A., Waters, D., Becker, T., Arendt, E., & Gastl, M. (2013). Protein modifications and metabolic changes taking place during the malting of common wheat (*Triticum aestivum* L.). *Journal of the American Society of Brewing Chemists*, 71, 153–160. <https://doi.org/10.1094/ASBCJ-2013-0613-01>
- Faltermaier, A., Waters, D., Becker, T., Arendt, E., & Gastl, M. (2014). Common wheat (*Triticum aestivum* L.) and its use as a brewing cereal – a review: Common wheat and its use as a brewing cereal. *Journal of the Institute of Brewing*, 120, 1–15. <https://doi.org/10.1002/jib.107>
- Ferreira, I., & Guido, L. (2018). Impact of wort amino acids on beer flavour: A review. *Fermentation*, 4, 23. <https://doi.org/10.3390/fermentation4020023>
- Fujita, A., Simsek, S., & Schwarz, P. B. (2020). Observations on the malting of ancient wheats: Einkorn, emmer and spelt. *Fermentation*, 6, 125. <https://doi.org/10.3390/fermentation6040125>
- Huerta-Zurita, R., Barr, J., Horsley, R. D., & Schwarz, P. B. (2020). Predicting malt fermentability in malting barley breeding lines. *Journal of the American Society of Brewing Chemists*, 78, 50–62. <https://doi.org/10.1080/03610470.2019.1670037>

- Hussain, A., Larsson, H., Kuktaite, R., Olsson, M., & Johansson, E. (2015). Carotenoid content in organically produced wheat: Relevance for human nutritional health on consumption. *International Journal of Environmental Research and Public Health*, *12*, 14068–14083. <https://doi.org/10.3390/ijerph121114068>
- Jaradat, A. (2013). Wheat landraces: A mini review. *Emirates Journal of Food and Agriculture*, *25*, 20. <https://doi.org/10.9755/ejfa.v25i1.15376>
- Jin, Y., Du, J., Zhang, K., & Guo, M. (2014). Relationships between the index of protein modification (Kolbach index) and hydrolytic enzyme production in a wheat malt: Index of protein modification and hydrolytic enzyme production. *Journal of the Institute of Brewing*, *120*, 201–206. <https://doi.org/10.1002/jib.136>
- Jin, Y., Zhang, K., & Du, J. (2008). Effects of wheat protein content on endosperm composites and malt quality. *Journal of the Institute of Brewing*, *114*, 289–293. <https://doi.org/10.1002/j.2050-0416.2008.tb00771.x>
- Krstanović, V., Mastanjević, K., Nedović, V., & Mastanjević, K. (2019). The influence of wheat malt quality on final attenuation limit of wort. *Fermentation*, *5*, 89. <https://doi.org/10.3390/fermentation5040089>
- Kuhn, M. (2016). *desirability: Function optimization and ranking via desirability functions*. R package version 2.1 <https://CRAN.R-project.org/package=desirability>.
- Lenth, R. V. (2009). Response-surface methods in R, using rsm. *Journal of Statistical Software*, *32*, 1–17. <https://doi.org/10.18637/jss.v032.i07>
- Liu, M., Zhao, Q., Qi, F., Stiller, J., Tang, S., Miao, J., et al. (2018). Sequence divergence between spelt and common wheat. *Theoretical and Applied Genetics*, *131*, 1125–1132. <https://doi.org/10.1007/s00122-018-3064-z>
- Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P., & Makowski, D. (2021). performance: An R package for assessment, comparison and testing of statistical models. *Journal of Open Source Software*, *6*, 3139. <https://doi.org/10.21105/joss.03139>
- Malekjani, N., & Jafari, S. M. (2020). Food process modeling and optimization by response surface methodology (RSM). In S. Sevda, & A. Singh (Eds.), *Mathematical and statistical applications in food engineering* (1st ed., pp. 181–203). CRC Press. <https://doi.org/10.1201/9780429436963-13>.
- Manuel Pais-Chanfrau, J., Núñez-Pérez, J., del Carmen Espin-Valladares, R., Vinicio Lara-Fiallos, M., & Enrique Trujillo-Toledo, L. (2021). Uses of the response surface methodology for the optimization of agro-industrial processes. In P. Kayaroganam (Ed.), *Response surface methodology in engineering science*. IntechOpen. <https://doi.org/10.5772/intechopen.98283>.
- Mefleh, M., Conte, P., Fadda, C., Giunta, F., Piga, A., Hassoun, G., et al. (2019). From ancient to old and modern durum wheat varieties: Interaction among cultivar traits, management, and technological quality: From ancient to old and modern durum wheat varieties. *Journal of the Science of Food and Agriculture*, *99*, 2059–2067. <https://doi.org/10.1002/jsfa.9388>
- Methner, F.-J. (2018). *Raw materials: Adjuncts, barley, malt, hops and hop products: Collection of brewing analysis Methods of the mitteleuropäische brautechnische analysenkommission (MEBAK)*. Freising, Germany, 2018.
- Meussdoerffer, F. G. (2009). A comprehensive history of beer brewing. In H. M. Elinger (Ed.), *Handbook of brewing* (pp. 1–42). Wiley-VCH Verlag GmbH & Co. KGaA. <https://doi.org/10.1002/9783527623488.ch1>.
- Muñoz-Insa, A., Gastl, M., Zarnkow, M., & Becker, T. (2011). Optimization of the malting process of oat (*Avena sativa* L.) as a raw material for fermented beverages. *Spanish Journal of Agricultural Research*, *9*, 510. <https://doi.org/10.5424/sjar/20110902-002-10>
- Muñoz-Insa, A., Selciano, H., Zarnkow, M., Becker, T., & Gastl, M. (2013). Malting process optimization of spelt (*Triticum spelta* L.) for the brewing process. *LWT. Food Science and Technology*, *50*, 99–109. <https://doi.org/10.1016/j.lwt.2012.06.019>
- Myers, R. H., Montgomery, D. C., & Anderson-Cook, C. M. (2009). Response surface methodology: Process and product optimization using designed experiments. In *Wiley series in probability and statistics* (3rd ed.). Wiley.
- Nakagawa, S., Johnson, P. C. D., & Schielzeth, H. (2017). The coefficient of determination R² and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. *Journal of The Royal Society Interface*, *14*, Article 20170213. <https://doi.org/10.1098/rsif.2017.0213>
- Narziß, L. (2005). *Abriss der Bierbrauerei* (7th ed.). Weinheim: Wiley-VCH.
- Narziß, L., & Back, W. (2012). *Die bierbrauerei: Band 2, technologie der Würzebereitung* (8 ed.). Weinheim: Wiley-VCH.
- Olivoto, T., & Lúcio, A. D. (2020). metan: an R package for multi-environment trial analysis. *Methods in Ecology and Evolution*, *11*, 783–789. <https://doi.org/10.1111/2041-210X.13384>
- Pedersen, T. L. (2020). *patchwork: The composer of plots*. R package version 1.1.1 <https://CRAN.R-project.org/package=patchwork>.

- Petzoldt, T. (2017). *Data analysis with R selected topics and examples*. Retrieved from https://wwwpub.zih.tu-dresden.de/~petzoldt/elements_en.pdf. (Accessed 3 June 2022).
- Phiarais, B. P. N., Schehl, B. D., Oliveira, J. C., & Arendt, E. K. (2006). Use of response surface methodology to investigate the effectiveness of commercial enzymes on buckwheat malt for brewing purposes. *Journal of the Institute of Brewing*, 112, 324–332. <https://doi.org/10.1002/j.2050-0416.2006.tb00738.x>
- R Core Team. (2021). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. <https://www.R-project.org/>.
- Rani, H., & Bhardwaj, R. D. (2021). Quality attributes for barley malt: “The backbone of beer. *Journal of Food Science*, 86, 3322–3340. <https://doi.org/10.1111/1750-3841.15858>
- Titze, J., Faltermaier, A., Schnitzenbaumer, B., Gastl, M., Becker, T., Ilberg, V., et al. (2013). Theoretical study on a statistical method for the simple and reliable pre-selection of wheat malt types for brewing purposes based on generally accepted quality characteristics. *Journal of the American Society of Brewing Chemists*, 71, 67–75. <https://doi.org/10.1094/ASBCJ-2013-0407-01>
- Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S* (4th ed.). New York: Springer, ISBN 0-387-95457-0.
- Wang, Z., Jiang, X., Zhang, Y., Du, Z., Feng, J., Quan, W., et al. (2022). Identification and validation of a major quantitative trait locus for adult plant resistance against leaf rust from the Chinese wheat landrace Bai qimai. *Frontiers of Plant Science*, 13, Article 812002. <https://doi.org/10.3389/fpls.2022.812002>
- Wang, Y., Liang, F., Guan, F., Yao, F., Long, L., Zhao, X., et al. (2021). Molecular mapping and analysis of an excellent quantitative trait loci conferring adult-plant resistance to stripe rust in Chinese wheat landrace gaixianguangtoumai. *Frontiers of Plant Science*, 12, Article 756557. <https://doi.org/10.3389/fpls.2021.756557>
- Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. New York: Springer-Verlag, 2016.
- Wolf-Hall, C. E. (2007). Mold and mycotoxin problems encountered during malting and brewing. *International Journal of Food Microbiology*, 119, 89–94. <https://doi.org/10.1016/j.ijfoodmicro.2007.07.030>
- Xu, X., Feng, J., Fan, J., Liu, Z., Li, Q., Zhou, Y., et al. (2018). Identification of the resistance gene to powdery mildew in Chinese wheat landrace Baiyouyantiao. *Journal of Integrative Agriculture*, 17, 37–45. [https://doi.org/10.1016/S2095-3119\(16\)61610-6](https://doi.org/10.1016/S2095-3119(16)61610-6)
- Zarnkow, M., Keßler, M., Burberg, F., Back, W., Arendt, E. K., & Kreis, S. (2007). The use of response surface methodology to optimise malting conditions of proso millet (*Panicum miliaceum* L.) as a raw material for gluten-free foods. *Journal of the Institute of Brewing*, 113, 280–292. <https://doi.org/10.1002/j.2050-0416.2007.tb00288.x>
- Zeven, A. (1998). Landraces: A review of definitions and classifications. *Euphytica*, 104, 127–139. <https://doi.org/10.1023/A:1018683119237>

5. Supplementary data

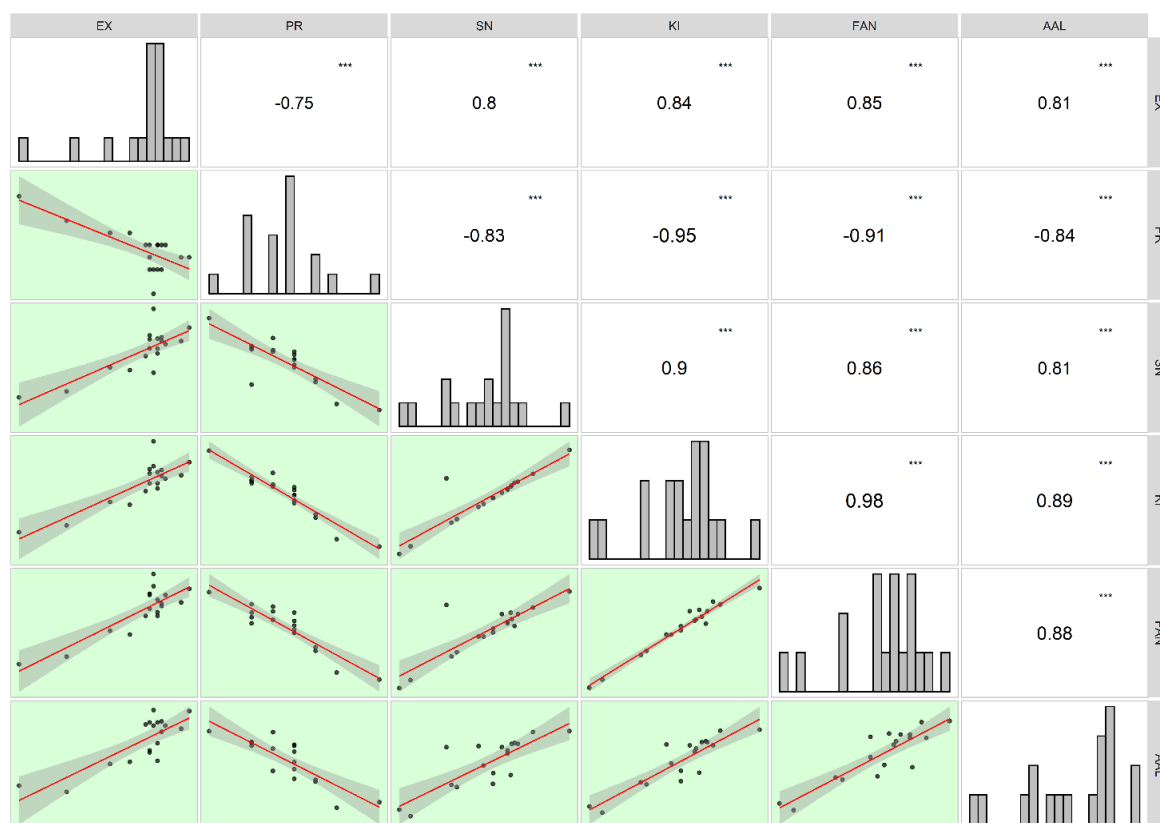


Fig. S1. Graphical and numerical visualization of the correlation matrix based on Pearson correlation method. The upper triangular section shows the correlation coefficients (r) of the malt quality traits. Significant correlation is indicated as follows: '***' 0.001. The lower triangular area shows bivariate scatterplots with a fitted line. EX: extract; PR: malt protein; SN: soluble nitrogen; KI: Kolbach index; FAN: free amino nitrogen; AAL: apparent attenuation limit.

Table S1.

R packages utilised for data analysis and graphical representation.

R package	Brief description
'utils', 'base', 'stats'	R: A language and environment for statistical computing (R Core Team, 2021)
'rsm'	Response-Surface Methods in R, Using rsm (Lenth, 2009)
'performance'	An R Package for Assessment, Comparison and Testing of Statistical Models (Lüdecke, Ben-Shachar, Patil, Waggoner, & Makowski, 2021)
'MASS'	Support functions and datasets (Venables & Ripley, 2002)
'desirability'	Function Optimization and Ranking via Desirability Functions (Kuhn, 2016)
'ggplot2'	Elegant Graphics for Data Analysis (Wickham, 2016)
'ggthemes'	Extra Themes, Scales and Geoms for 'ggplot2' (Arnold, 2021)
'patchwork'	The Composer of Plots (Pedersen, 2020)
'metan'	An R package for multi-environment trial analysis (Olivoto & Lúcio, 2020)

Table S2

Levels of the malting parameters, in coded and natural units, and other malt quality traits that presented a significant lack of fit (not considered for further analysis).

run.order	std.order	xA	xB	xC	A	B	C	Soluble nitrogen (mg/100 g)	pH	Colour (EBC units)	Viscosity (mPa × s)	Viscosity 65 °C (mPa × s)
1	6	1	-1	1	6	12	46	765	6.07	2.6	1.83	2.09
2	2	1	-1	-1	6	12	40	740	6.09	2.6	1.86	2.10
3	8	1	1	1	6	18	46	962	6.01	4.2	1.55	1.83
4	4	1	1	-1	6	18	40	820	6.05	3.5	1.60	1.91
5	10	0	0	0	5	15	43	787	6.05	3.1	1.72	2.03
6	3	-1	1	-1	4	18	40	727	6.10	3.1	1.78	2.05
7	7	-1	1	1	4	18	46	829	6.04	3.1	1.70	2.00
8	17	0	0	-1	5	15	40	764	6.08	3.1	1.77	2.06
9	11	0	0	0	5	15	43	810	6.05	3.1	1.72	2.02
10	16	0	1	0	5	18	43	813	6.05	3.1	1.63	1.93
11	5	-1	-1	1	4	12	46	551	6.13	2.5	2.25	2.34
12	15	0	-1	0	5	12	43	657	6.11	2.6	2.05	2.35
13	14	1	0	0	6	15	43	868	6.05	3.2	1.64	1.94
14	18	0	0	1	5	15	46	644	6.04	3.4	1.70	1.99
15	12	0	0	0	5	15	43	802	6.05	3.0	1.71	2.01
16	13	-1	0	0	4	15	43	671	6.07	3.0	1.96	2.16
17	1	-1	-1	-1	4	12	40	522	6.15	2.5	2.22	2.29
18	9	0	0	0	5	15	43	802	6.04	3.0	1.72	2.01

A: germination time (d); B: germination temperature (°C); C: degree of steeping (g/100 g); Viscosity: measured in Congress wort; Viscosity 65 °C: measured in isothermal 65 °C mash.

Table S3

Model comparison for each response.

	MODEL	R ²	Adjusted R ²	RMSE	Sigma	AIC	BIC
Extract	Linear	0.580	0.490	0.648	0.735	45.488	49.94
	Two-way interaction	0.789	0.674	0.460	0.588	39.109	46.232
	Quadratic	0.956	0.906	0.211	0.316	17.054	26.848
KI	Linear	0.88	0.855	1.729	1.961	80.801	85.253
	Two-way interaction	0.924	0.882	1.38	1.765	78.678	85.801
	Quadratic	0.992	0.982	0.459	0.689	45.079	54.873
FAN	Linear	0.773	0.724	8.846	10.031	139.561	144.013
	Two-way interaction	0.851	0.769	7.177	9.181	138.035	145.158
	Quadratic	0.976	0.949	2.866	4.3	110.992	120.786
AAL	Linear	0.778	0.73	1.076	1.221	63.735	68.187
	Two-way interaction	0.846	0.761	0.897	1.148	63.176	70.299
	Quadratic	0.867	0.718	0.832	1.247	66.439	76.233

The chosen models are highlighted in bold. RMSE: Root Mean Squared Error; Sigma or RSE: Residual Standard Error; AIC: Akaike's Information Criteria; BIC: Bayesian Information Criteria. KI: Kolbach index; FAN: free amino nitrogen; AAL: apparent attenuation limit.

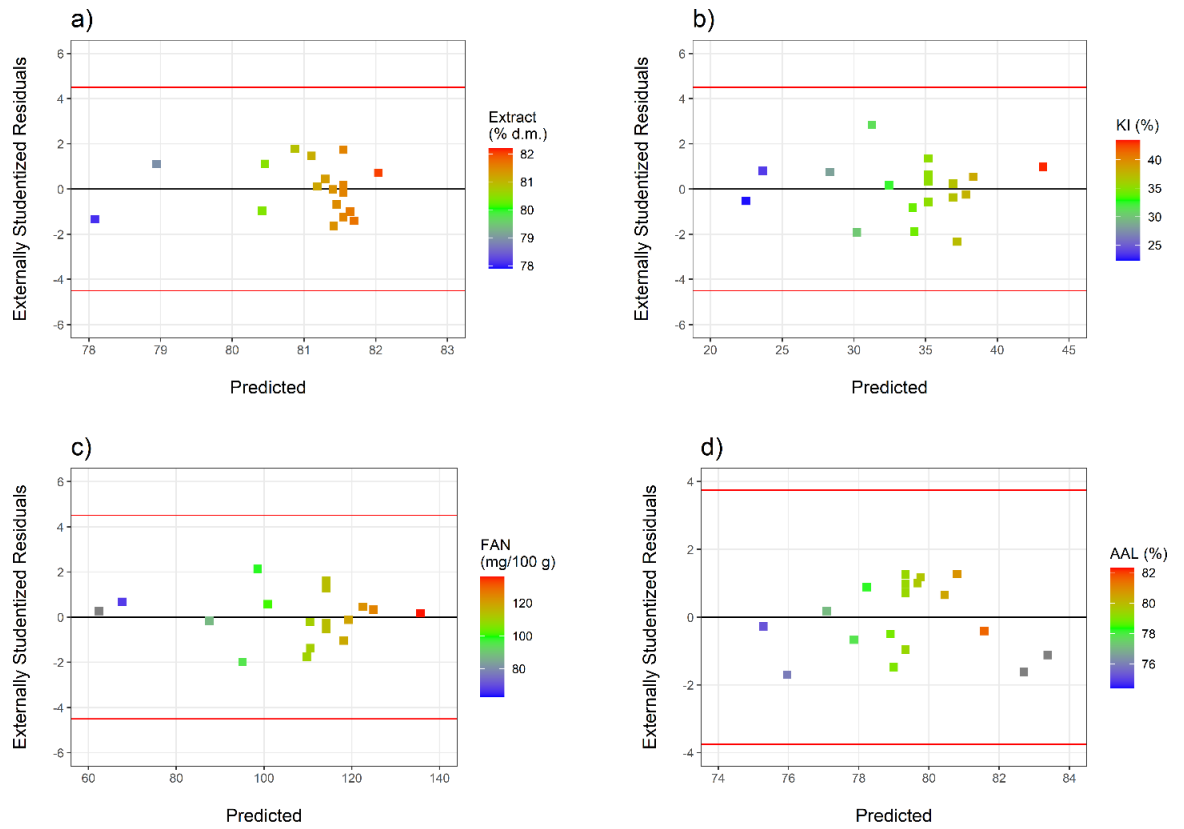


Fig. S2. Plot of externally studentized residuals versus predicted values. Different letters in each subplot refer to a specific response: a) extract; b) KI: Kolbach index; c) FAN: free amino nitrogen; d) AAL: apparent attenuation limit. The colour palette depicted in the legend refers to increasing values for each predicted response, from blue to red colour.

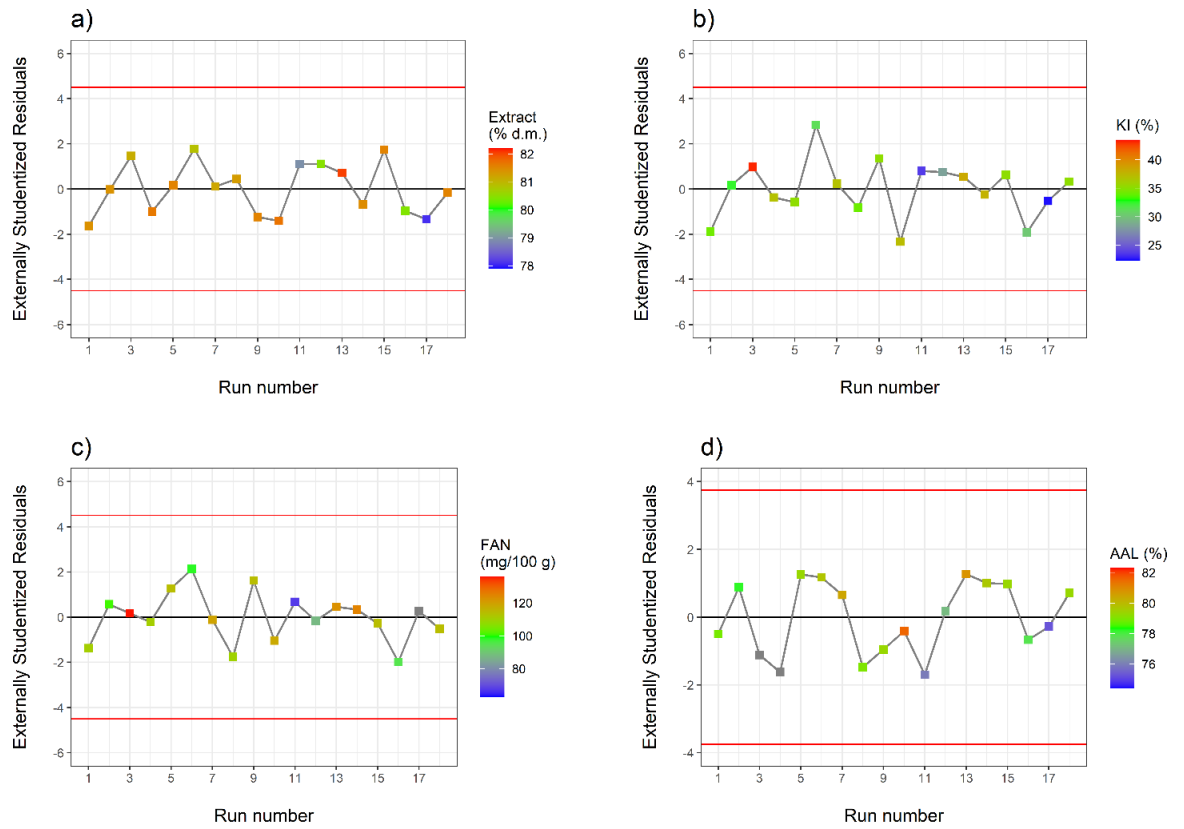


Fig. S3. Plot of externally studentized residuals vs experimental run order. Different letters in each subplot refer to a specific response: a) extract; b) KI: Kolbach index; c) FAN: free amino nitrogen; d) AAL: apparent attenuation limit. The colour palette depicted in the legend refers to increasing values for each predicted response, from blue to red colour.

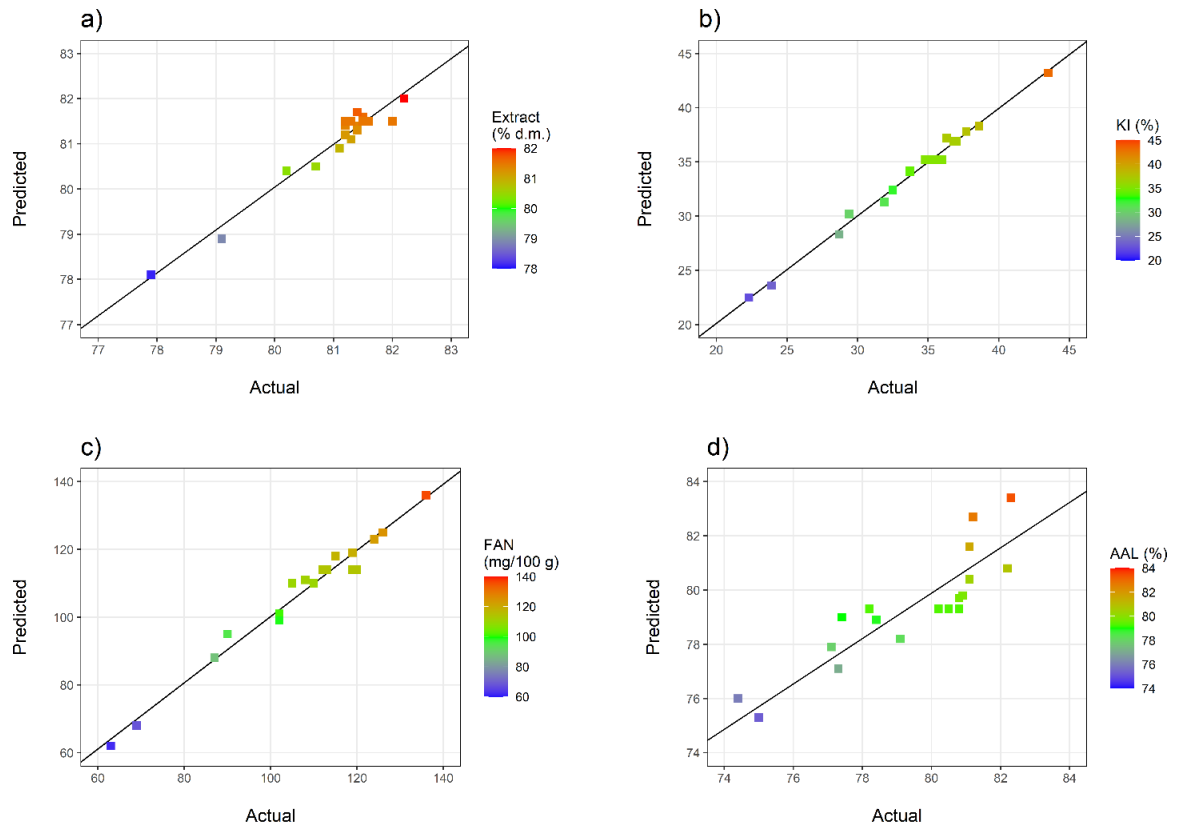


Fig. S4. Plot of predicted versus actual values. a) extract; b) KI: Kolbach index; c) FAN: free amino nitrogen; d) AAL: apparent attenuation limit. The colour palette depicted in the legend refers to increasing values for each predicted response, from blue to red colour.

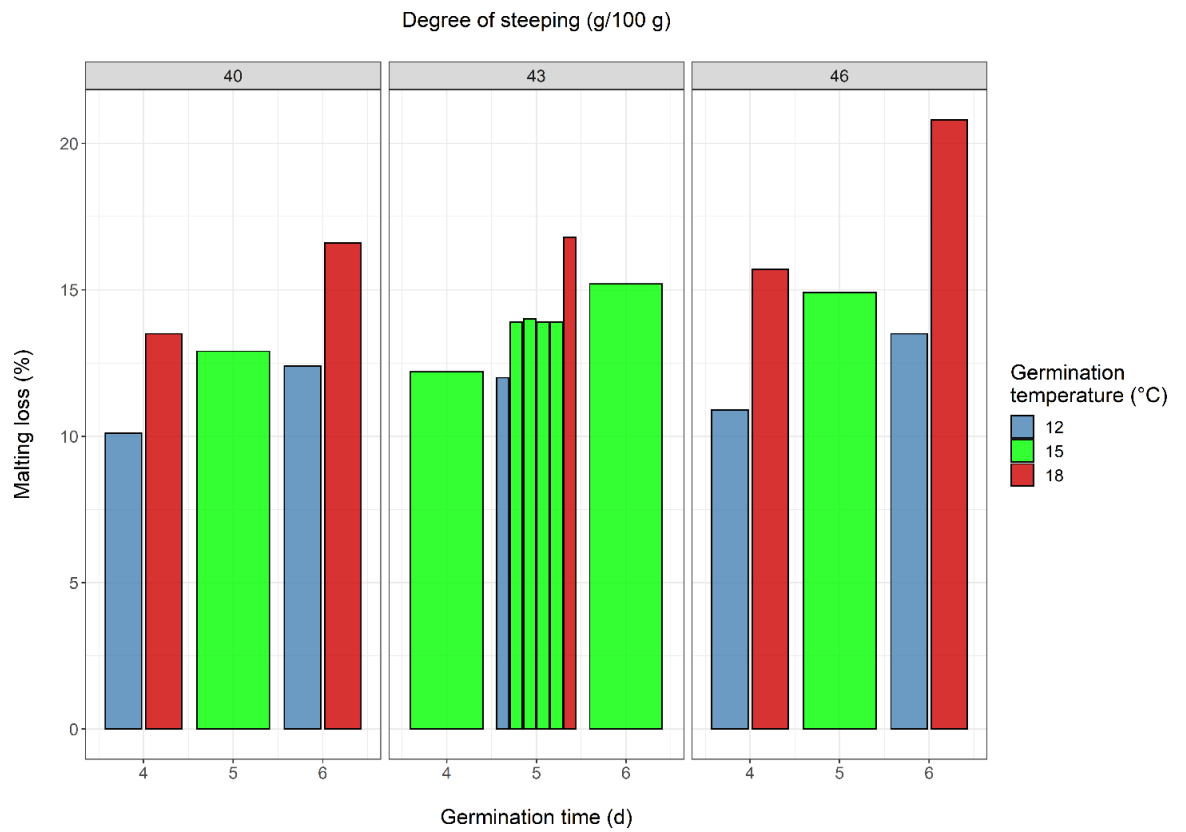


Fig. S5. Influence of germination time (d), germination temperature (°C) and degree of steeping (g/100 g) on malting losses (%). The graph is split into three sub-plots for each degree of steeping. Different colours refer to distinct levels of germination temperatures as specified in the legend.

Capitolo 5

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Multi-Response Optimization of the Malting Process of an Italian Landrace of Rye (*Secale cereale* L.) Using Response Surface Methodology and Desirability Function Coupled with Genetic Algorithm

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Abstract: Rye is used in some applications in the food and beverage industry and for the preparation of functional foods. It is an interesting raw material in malting and brewing due to its characteristic contribution to the beer's color, turbidity, foam and aroma. The aim of this work was to optimize the micro-malting process of a rye landrace. The response surface methodology (RSM) was applied to study the influence of three malting parameters (germination time, germination temperature and degree of steeping) on the quality traits of malted rye. Long germination times at high temperatures resulted in an increase in the extract and Kolbach index. The model for the apparent attenuation limit showed a particular pattern, whereby time and temperature inversely influenced the response. The lowest viscosities were determined in the worts produced from highly modified malts. Optimization of the variables under study was achieved by means of a desirability function and a genetic algorithm. The two methodologies provided similar results. The best combination of parameters to optimize the malting process on the rye landrace under study was achieved at 6 days, 12 °C and 44 g/100 g.

Keywords: rye; landrace; response surface methodology; malting optimization; desirability function; genetic algorithm

1. Introduction

In recent years, the evolution and competition between beer multinational corporations and craft breweries have become increasingly intense [1]. Local companies rely on marketing strategies that emphasize the origin of raw materials and the link with the territory. Indeed, according to a study on the Italian craft beer sector, more than 70% of the surveyed breweries included information on the origin of the raw materials [2]. Such an approach is based on the concept of neolocalism, defined as “a conscious effort by businesses to foster a sense of place based on attributes of their community” [3]. Research is interested in the reintroduction of traditional rye varieties that enable high yields in marginal and poor soils [4]. Landraces are autochthonous and genetically related varieties that indeed exhibit stable yields in low-input environments over time [5]. The trend towards the rediscovery of local genetic resources can also be traced to the Calabria region (southern Italy). Rye landraces are typically cultivated in the mountainous areas of the region, above an altitude of 750 m. Interestingly, this rustic cereal is known by different names in the local dialects, e.g., ‘*iermunu*’ or ‘*granuiermanu*’, words that have a peculiar assonance with Germany, the country from which rye was allegedly originally

imported [6]. Local craft breweries are strongly interested in locally grown raw materials and beers containing rye are part of their special releases. However, malts derived from local grains are essentially unavailable.

Rye (*Secale cereale* L.) is a species belonging to the *Poaceae* family. It is genetically close to wheat to the extent that the commercial hybrid Triticale (x *Triticosecale* Wittmack) is derived from them [7]. The domestication and spread of rye as a crop in human civilization are shrouded in mystery. Native to the Anatolian peninsula, it initially spread to central Europe as a weedy plant [8]. A variety of controversial theories have tried to shed light on the species. Anyhow, it is generally agreed that it was not among the first cereals cultivated at the dawn of agriculture [9]. Considered a minor cereal, it is second only to wheat for the production of bread and other bakery products [10]. Its cultivation is generally restricted to cold climates and harsh environments where other cereals would struggle to thrive [11]. Total world production in 2020 was 15,022,273 tons, with Germany, Poland and the Russian Federation among the top producers. From 2010 to 2020, Italy's rye production was fluctuating with an average yield of 13,030.5 tons [12]. Rye has a carbohydrate content varying between 66% and 80%, while total protein ranges from 6.5 to 14.5% [13]. It represents an outstanding source of dietary fiber, bioactive compounds [14], polyphenols, flavonoids and thiols, whose contents are strongly affected by genotype [15]. Health benefits related to its consumption as a whole grain have been reported to improve gut function [16]. Secondary metabolites known as benzoxazinoids, which may exert antimicrobial and anticarcinogenic roles [17], have been detected in wheat- and rye-based beers [18]. Remains of unmalted rye were found at an archaeological site in Berlin, along with oats and germinated barley. Albeit a matter of conjecture, this could still suggest that rye was used in brewing in the Middle Ages [19]. Nowadays, rye is mainly used in the beverage industry for beer [20] and whisky production [21]. In brewing, it mainly serves as an adjunct, that is to say, a source of fermentable extract at a reduced cost [22]. It is the key ingredient of traditional drinks such as kvass, a Russian fermented beverage made of rye bread [23], and historical beers including the Bavarian Roggenbier and the Finnish sahti, a farmhouse ale flavored with juniper branches and fermented with baker's yeast [24]. Moreover, rye enters the grist of beer styles such as Belgian Pale Ale [25], Saison and Specialty India Pale Ale (Rye IPA), sought after for its spicy and pungent aroma as a complementary ingredient to barley malt [26]. The malting of rye is similar to that of wheat, although its germination tends to be faster compared to other seeds [27]. Rye grain shares some features with wheat such as rapid water absorption and the potential to give more extract than barley. However, the grain is more prone to damage due to the lack of husks [28].

Information on the malting of rye is limited compared to that of wheat and barley. In this study, the response surface methodology was applied to the micro-malting of rye to investigate how three malting parameters (germination time, germination temperature, and degree of steeping) can influence the quality of derived malts. RSM consists of a set of mathematical and statistical tools to develop and optimize processes in a wide variety of research fields [29]. Appropriate mathematical models are defined to investigate the relationship between process parameters and responses of interest to determine optimum conditions [30]. The final stage of this methodology may consist of the simultaneous optimization of several conflicting objects. Widely used methods include the desirability function and the genetic algorithm. The former was originally proposed by Harrington (1980), and his approach was later modified by Derringer and Suich (1984) using a discontinuous function [31]. Theoretical and practical research on genetic algorithms based on biological evolution has been conducted for over 40 years by John H. Holland and his research team since the mid-1960s [32]. Genetic algorithms can be successfully combined with the desirability function using models derived from response surface analysis [33,34]. A genetic algorithm consists of at least the following three operators: reproduction, crossover and mutation. A population composed of individuals or chromosomes exchanges information through reproduction. Crossover and mutation operators are involved in the creation of the new generations. Thus, the offspring will result from the combination of information from both parents [35]. Selection of the fittest is based on an objective function (fitness

function), i.e., a measure of goodness to be increased. Thus, strings with greater values will have a higher likelihood of transferring their information to subsequent generations, similar to the adaptive process of natural selection and survival of the fittest [36]. To the best of our knowledge, this is the first work in which the malting process of rye has been optimized using two different multi-objective optimization techniques.

2. Materials and Methods

2.1. Raw Material

A landrace of rye (*Pollino*) with a moisture of 11.0% and a protein content of 14.8% d.m. was used in the micro-malting experiments. The raw material was supplied by a local farm located in the Pollino UNESCO Global Geopark in Calabria region (southern Italy). The initial germinative energy of the grain was 90%, therefore, samples of rye were subjected to 40 °C for a week, in a temperature-controlled chamber, in an attempt to increase it. Afterward, the germination energy remained almost identical. For this reason, the rye samples were malted without any preliminary treatment. Grain samples showed great variability in size, being mostly small and tapered. The results of the sieving test, according to EBC method 3.11.1 for barley, were as follows: grade I, 8.7; grade II, 30.1; rejects, 61.2. Before malting, samples were cleaned and sieved using a sieve machine with sorting sieves (SLN Sample Cleaner Pfeuffer). Both fractions I and II (large and small seeds, respectively) were malted. Although it is recommended to use similar-sized grains to achieve the most uniform malting, in this case, smaller and larger grains were malted together as they were representative of the starting material.

2.2. Experimental Design

Response surface methodology was used to generate the experimental domain and for data analysis. A face-centered central composite design (FCCD), broadly used in research on micro malting optimization [37–39], was chosen to set up the experiments. This experimental design is shaped in the form of a cube with the axial points located at the center of the cube faces, with $\alpha = 1$. The malting process parameters were selected as germination time (d), germination temperature (°C) and degree of steeping (g/100 g). The germination time (A) varied from 4 to 6 days, the germination temperature (B) from 12 to 18 °C, the degree of steeping (C) from 40 to 46 g/100 g. The central points were defined at 5 days, 15 °C and 43 g/100 g. Factor levels were converted in coded units ranging from -1 to +1. Malting parameters into coded form were defined as follows: x_A for the germination time; x_B for the germination temperature; x_C for the degree of steeping. The experimental design consisted of 8 corner points, 4 center points and 6 axial or star points (α), for a total of 18 randomized runs (Table 1). The analyzed responses were as follows: extract, Kolbach index, apparent attenuation limit, viscosity measured in Congress wort and isothermal mash.

2.3. Micro-Malting Plan

For each combination of process parameters, 1 kg of rye was malted in the micromalting plant of the Research Centre Weihenstephan for Brewing and Food Quality (Freising, Germany), Technical University of Munich (TUM). Due to technical issues, the experiment was completed by adopting the same procedure at the malting facility of the Chair of Brewing and Beverage Technology, Technical University of Munich (TUM). The steeping process was conducted as defined as follows: two stages of imbibition in water of 5 and 4 h were followed by air rests of 19 and 20 h, respectively, for a total of 48 h. The steeping temperature was set according to the experimental design. The moisture level for each sample was brought to the three different target levels on the first day of germination according to the experimental design. It was then maintained at the desired level by spraying water on the following days. Germination time was varied according to the experimental scheme. The samples were subjected to gentle rotation throughout the process to avoid packing. Once the germination step was completed, the samples were subject to the following kilning schedule: 50 °C for 16 h, 60 °C for 1 h, 70 °C for 1 h and 80

°C for 5 h. Afterward, dry rootlets were removed and the different malts were stored for one week prior to analysis. All malt samples presented a moisture content of less than 5%.

Table 1. Face-centered design with coded and natural variables.

Run Order	x _A	x _B	x _C	A	B	C
1	-1	1	1	4	18	46
2	0	0	0	5	15	43
3	-1	1	-1	4	18	40
4	0	0	-1	5	15	40
5	1	1	-1	6	18	40
6	0	0	0	5	15	43
7	0	0	0	5	15	43
8	-1	0	0	4	15	43
9	1	-1	-1	6	12	40
10	1	0	0	6	15	43
11	0	1	0	5	18	43
12	1	1	1	6	18	46
13	0	-1	0	5	12	43
14	0	0	1	5	15	46
15	-1	-1	-1	4	12	40
16	-1	-1	1	4	12	46
17	0	0	0	5	15	43
18	1	-1	1	6	12	46

2.4. Chemical Analyses

Malts and worts were analyzed in duplicate (n = 2) according to the methods of the Mitteleuropäische Brautechnische Analysenkommision (Central European Brewing Technology Commission or MEBAK) [40], using Congress and isothermal 65 °C mash programs. The mean of each measurement has been reported. Before malting, some quality attributes of grain were evaluated. The moisture content of the raw material was measured by means of R-110.40.020 method (Drying Oven Method) as difference in mass before and after drying, while the R-110.29.612 method (AUBRY Method) was used to assess the germinative energy. The nitrogen content was determined by R-110.41.030 according to Kjeldahl method. The protein content was derived by multiplying the nitrogen content by the conversion factor of 5.83 [41]. The moisture content of malts was determined by R-200.18.020, as mass loss over a standardized drying procedure. Soluble nitrogen and protein content were measured according to R-205.11.030 and R-200.20.030, respectively, according to Kjeldahl method. Kolbach index was calculated as the ratio of soluble to total nitrogen, according to R-205.12.999 method. The extract was assessed on the Congress mash by R-205.01.080 method. Apparent attenuation limit (AAL) was measured according to R- 205.16.080 method (Fermentation Tube Method). Viscosity was measured in Congress wort and isothermal (65 °C) wort according to R-205.10.282 method using a microviscometer from Anton Paar.

2.5. Statistical Analysis

Data analysis was performed in RStudio, a free software environment for statistical computing and graphics, R version 4.1.0 (2021-05-18). Response surface analysis was carried out using ‘rsm’ package [42]. The ‘graphics’ package [43] was used to visualize the response surfaces. Model checking adequacy was conducted by means of ‘performance’ [44], and ‘mass’ packages [45]. Data visualization was realized using basic functions of R, ‘ggplot’ [46], ‘viridis’ [47] and ‘patchwork’ [48] packages. Multi-criteria optimization was conducted using ‘desirability’ [31] and ‘GA’ [35] packages. Malting at 6 days, 15 °C and 43 g/100 g (run No. 10) was not possible due to technical issue related to the malting plant. The handling of missing data generally consists of the following two strategies: analyzing an incomplete dataset or resorting to strategies such as multiple imputation. In the latter case, missing data are replaced with new values on the basis of experimental observations [49]. In order to check whether missing values could alter the

predicted results and thus influence the goodness of the method, missing values were replaced by means of multivariate imputation by chained equations (data not shown) using the ‘mice’ package [50]. It was possible to conclude that the results with and without imputation were comparable. In light of this, it was preferred to keep the missing values during the data analysis.

3. Results and Discussion

3.1. Model Fitting and Adequacy Evaluation

The first important step in the response-surface analysis is to set up the relationship between natural and coded variables, as given in the following equations below:

$$xA = (A - 5)/1 \quad (1)$$

$$xB = (B - 15)/3 \quad (2)$$

$$xC = (C - 43)/3 \quad (3)$$

where the value of a coded variable is equal to the difference between the uncoded form and its center points, divided by half the variable’s range. Coding ensures that all input variables are dimensionless and vary over the same interval, so as to guarantee a feasible interpretation of the surface analysis [42]. Quadratic or second-order models were defined to approximate true response functions. The models’ performance was compared on the basis of statistical fit indices and the significance of the models’ coefficients. The Akaike information criterion (AIC) and Bayesian information criterion (BIC) are widely used as indicators to select the most suitable models. In brief, the fitted models with the lowest AIC and BIC and the highest adjusted R squared were selected for data analysis (Table S1). The only exception was the model for the AAL, where only one interaction term was found to be statistically significant. Nevertheless, it was decided to choose the quadratic model, taking into account the relevant values for the adjusted R squared, AIC and BIC, as well as the performance of the model (Table S1).

The linear, interaction and quadratic terms were found to be variously significant for the preliminary fitted models (Figure 1).

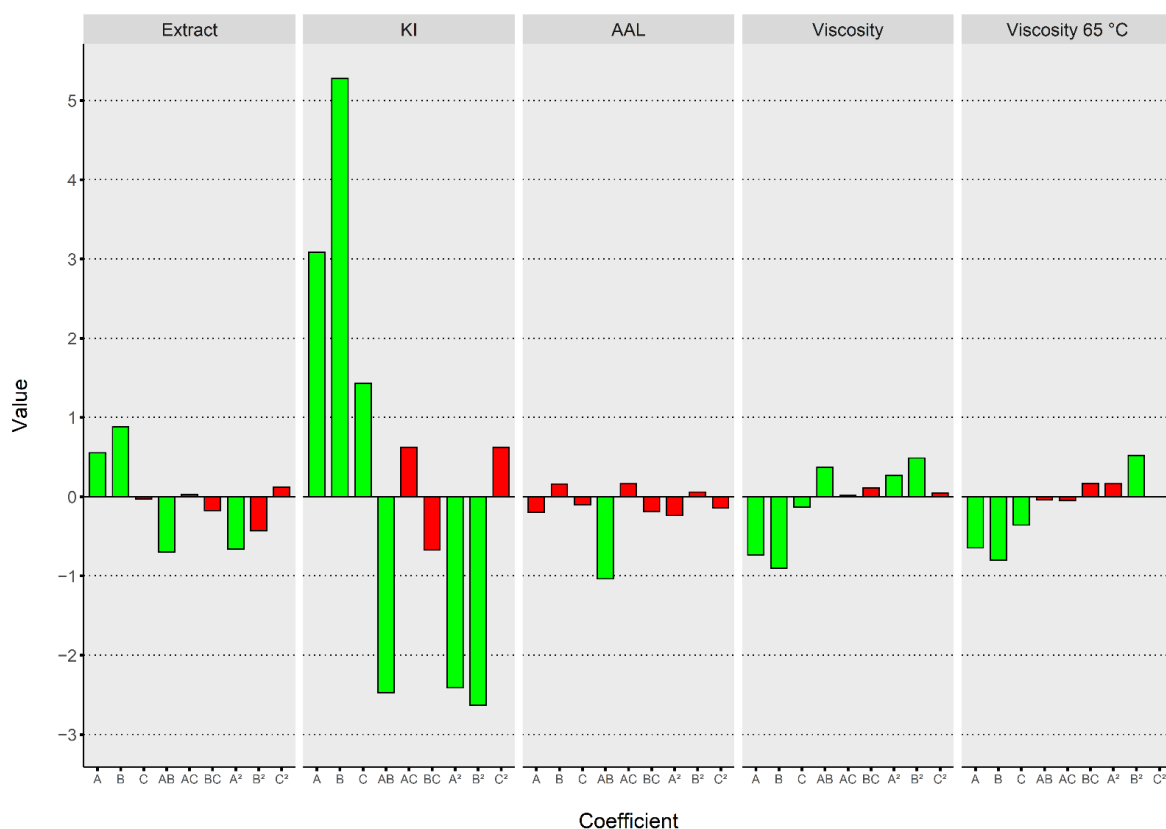


Figure 1. Comparison of coefficient estimates in terms of coded factors. The colors of the bars indicate the significance of each term: green for significant terms, red for non-significant terms. KI, Kolbach index; AAL, apparent attenuation limit; A, germination time; B, germination temperature; C, degree of steeping.

At first glance, the process parameters exerted different influences on the responses under study. In particular, the KI variable was the most affected overall, with the germination temperature exerting a large positive impact. All linear terms had a negative impact on viscosities. All models resulted in significance based on the F-values. In developing models within the adopted methodology, attention must be paid to the lack of fit test, a statistical indicator used to check the model's adequacy in predicting response surfaces [51]. Fitted models presented a non-significant lack of fit, so they were considered suitable for making predictions and drawing conclusions from the response surfaces. Table 2 shows the outcomes of the analysis of variance for all selected models.

Table 2. Analysis of Variance (ANOVA) of the fitted models.

Response	Source of Variation	Df ¹	Sum Sq ²	Mean Sq ³	F Value	p-Value
Extract	Model	5	18.05	10.9843	48.31	***
	Residuals	11	0.8220	0.0747		
	Lack of fit	2	0.0170	0.0085	0.0949	ns
	Pure error	9	0.8050	0.0894		
KI ⁴	Model	9	513.82	171.275	32.31	***
	Residuals	7	12.37	1.767		
	Lack of fit	4	10.55	2.637	4.3465	ns
	Pure error	3	1.82	0.607		
AAL ⁵	Model	9	10.20	3.39975	18.65	***
	Residuals	7	0.4254	0.06078		
	Lack of fit	4	0.3654	0.09136	4.5680	ns
	Pure error	3	0.0600	0.02000		
Viscosity	Model	8	16.2291	5.7671	106.7	***
	Residuals	8	0.1521	0.0190		
	Lack of fit	5	0.1386	0.0277	6.172	ns
	Pure error	3	0.0135	0.0045		
Viscosity 65 °C	Model	7	13.0247	5.4064	34.32	***
	Residuals	9	0.4879	0.0542		
	Lack of fit	6	0.3979	0.0663	2.2107	ns
	Pure error	3	0.0900	0.0300		

¹ Df, degree of freedom; ² Sum Sq, sum of squares; ³ Mean sq, Mean Square; ⁴ KI, Kolbach index; ⁵ AAL, apparent attenuation limit. The symbols ***, ns, indicate the following levels of significance for each ANOVA model: 0.001, not significant.

All ANOVA assumptions were confirmed throughout the analysis of externally studentized residuals and diagnostic plots. Residuals were found to be normally distributed, so the assumption of normality was fulfilled. No particular trend was found for residuals against predicted values (Figure S1) and runs (Figure S2). The points resulting from the combination of predicted and observed values were randomly distributed and close to the fitted lines (Figure S3). This is an indication of good prediction for all analyzed models, as also suggested by the values of the coefficients of determination that resulted close to the unit. The comparison between actual and predicted outcomes is reported in Table 3.

Table 3. Comparison between actual and predicted responses using RSM.

Run order	Extract (% d.m. ¹)		KI ² (%)		AAL ³ (%)		Viscosity (mPa × s)		Viscosity 65 °C (mPa × s)	
	Actual	Predicted	Actual	Predicted	Actual	Predicted	Actual	Predicted	Actual	Predicted
1	83.2	83.4	60.6	59.7	73.5	73.7	4.99	4.97	6.93	6.84
2	83.4	83.4	60.3	59.4	73.0	73.0	4.67	4.77	6.36	6.46
3	83.5	83.4	59.6	59.5	74.7	74.6	4.92	5.05	7.13	7.12
4	83.3	83.4	57.3	58.6	72.7	73.0	5.04	4.90	6.88	6.82
5	83.5	83.1	60.6	59.4	71.8	71.8	4.29	4.30	5.99	5.82
6	83.3	83.4	60.4	59.4	72.9	73.0	4.69	4.77	6.12	6.46
7	83.1	83.4	59.3	59.4	72.9	73.0	4.71	4.77	6.54	6.46
8	82.3	82.2	53.2	53.9	73.1	73.0	5.82	5.79	7.42	7.12
9	82.5	82.7	51.8	52.5	73.3	73.2	5.56	5.59	7.77	7.84
10	na ⁴	na ⁴	na ⁴	na ⁴	na ⁴	na ⁴	na ⁴	na ⁴	na ⁴	na ⁴
11	83.9	83.8	60.4	62.0	73.3	73.3	4.55	4.35	5.94	6.28
12	82.7	83.1	61.7	62.2	71.5	71.5	4.19	4.28	5.42	5.34
13	82.1	82.1	52.4	51.5	73.0	72.9	6.00	6.16	8.14	7.89
14	83.8	83.4	62.0	61.4	73.2	72.8	4.63	4.63	6.17	6.10
15	80.3	80.2	43.3	42.6	71.8	71.8	7.89	7.81	8.93	8.99
16	80.1	80.2	44.6	45.6	71.6	71.7	7.29	7.29	7.89	8.03
17	83.2	83.4	58.8	59.4	73.2	73.0	4.82	4.77	6.38	6.46
18	83.0	82.7	58.0	57.9	73.5	73.7	5.25	5.13	6.70	6.68

¹ d.m., dry matter; ² KI, Kolbach index; ³ AAL, apparent attenuation limit; ⁴ na, not available.

3.2. Extract

The extract is a measure of malt yield [28] used to estimate the number of soluble substances in the wort [52]. The extract values of rye and wheat malt are generally higher than those of barley malt [28]. Wang et al. (2018) reported average extract values comprised between 85.0 and 85.9% d.m. for germination times between 3 and 6 days, with no significant differences after three days of germination [53]. The observed values ranged from 80.1 to 83.9% d.m. The minimum extract was obtained after 4 days at 12 °C and 46 g/100 g, while the maximum was obtained after 5 days at 18 °C and 43 g/100 g, respectively. A similar extract (83.8% d.m.) was also reached after 5 days, at a lower temperature and a higher degree of steeping (15 °C and 46 g/100 g, respectively). Values beyond 83.0% d.m. could be obtained at every level of germination time and degree of steeping at temperatures ranging between 15 °C and 18 °C. The quantity of extract measured after 6 days of germination showed a different trend depending on the temperature degree of the steeping combination, according to an inversely proportional relationship. Therefore, by raising the temperature from 12 to 18 °C with a simultaneous reduction of the degree of steeping from 46 to 40 g/100 g, an increase in the extract from 83 to 83.5% was observed. Indeed, it is well known that a gradual increase in the moisture content of the germinating seed enhances the embryo's growth rate and respiration, resulting in reduced extract content [28]. The best-fitted model describing the response was the following:

$$Extract = 83.3652 + 0.5636 \times xA + 0.8800 \times xB - 0.7000 \times xAxB - 0.5929 \times xA^2 - 0.4109 \times xB^2 \quad (4)$$

The adjusted R squared was high and close to the unit (0.937), and the lack of fit was not significant (p-value: 0.9103533). Germination time and temperature exerted a major role in the response. They both presented positive coefficients, which means increasing A or B determined a consequent increase in the predicted response. Nonetheless, their interaction was also significant. Its coefficient presented a negative sign, which means the effect of A on the response will decrease by raising B and vice versa. The quadratic coefficients for the same parameters were also significant. The degree of steeping did not exert any significant influence on the extract, and for this reason, it was not considered in the model. Figure 2 shows the 3D surface of the predicted response based on the fitted model. It was possible to identify a point near-maximum at about 5 days at 18 °C. Predicted extracts equal to 83.7% could be obtained for temperatures comprised between about 17 and 18 °C and for germination times ranging from 4.5 to 5.5 days.

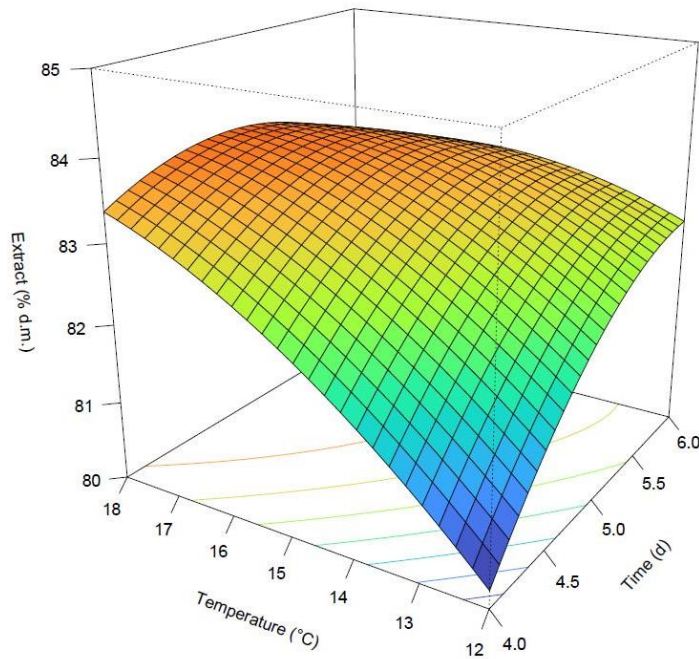


Figure 2. Surface plot of the predicted extract.

3.3. Kolbach Index

KI is defined as the ratio of soluble to total nitrogen and indicates the degree to which the malt is modified [40]. It is a measure of the proteolytic activity that occurs in malting. The recommended range for barley malt for short-infusion mashing is 38–42% [54]. The highest soluble nitrogen contents were found at higher temperatures for long germination times (data not shown), as also reported in Hübner & Arendt (2010) [55]. The minimum observed value (43.3%) was attained at 4 days, 12 °C and 40 g/100 g, while the maximum (62.0%) was attained at 5 days, 15 °C and 46 g/100 g. Overall, lower values of KI have been detected if compared with the results reported in Wang et al. (2018) [53]. These differences can presumably be attributed to the different experimental designs used as well as genotypic differences. Increasing the germination time from the lowest to the highest level while keeping the temperature and degree of steeping at a constant level of 12 °C and 40 g/100 g, respectively, determined an increase in KI from 43.3 to 51.8%. A quadratic model described the response as follows:

$$KI = 59.3654 + 3.0837 \times xA + 5.2800 \times xB + 1.4300 \times xC - 2.4750 \times xAxB + 0.6250 \times xAxC - 0.6750 \times xBxC - 2.4125 \times xA^2 - 2.6308 \times xB^2 + 0.6192 \times xC^2 \quad (5)$$

The adjusted R squared was equal to 0.946, which means most of the variability observed was explained by the selected model. The lack of fit was not significant (p-value: 0.128669). All the linear terms were significant, with the germination temperature exerting the strongest influence on the response (Figure 1). The interaction term between germination time and the temperature was also significant, and the coefficient presented a negative sign. They both contributed to influencing the KI response; for this reason, the effect of A on KI resulted less strong with a concurrent increase in B and vice versa. There was a discrepancy regarding the combination of experimental settings allowing to reach the maximum response if observed and predicted values

were compared. In fact, the maximum KI (62.2%) was predicted at 6 days, 18 °C and 46 g/100 g. Nonetheless, an area of maximum predicted response (>62.0%) has been predicted by the fitted model in the range 5–5.5 days and 17–18 °C when the degree of steeping was held at a constant level of 43 g/100 g, as depicted in Figure 3.

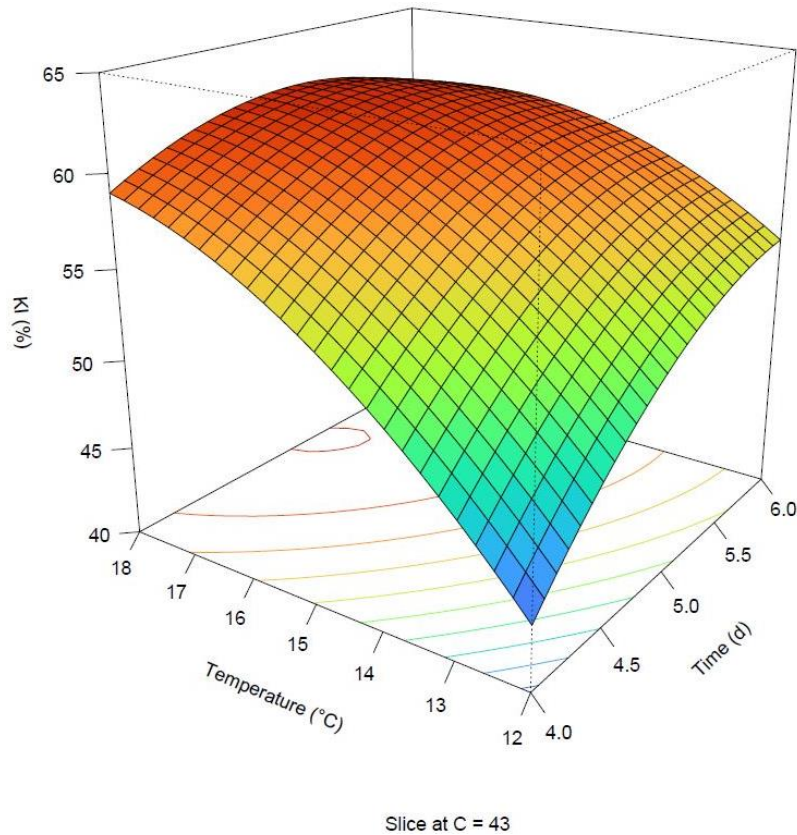


Figure 3. Surface plot of the predicted Kolbach index. The degree of steeping is held at 43 g/100 g.

3.4. Apparent Attenuation Limit

The apparent attenuation limit measured in the Congress wort represents the difference in extract before and after fermentation [40]. In particular, the term attenuation refers to the decrease in the specific gravity of the wort and thus its sugar content after fermentation [28]. The recommended value for barley malt is over 80% [56]. The observed responses were in the range of 71.5–74.7%. The minimum value was reached at 6 days, 18 °C and 46 g/100 g, while the maximum value was reached at 4 days, 18 °C and 40 g/100 g. An average AAL of 73.0% was obtained at the central points of the experimental region. The second-order model describing the AAL response is shown as follows:

$$AAL = 73.0460 - 0.1990 \times xA + 0.1600 \times xB - 0.1000 \times xC - 1.0375 \times xAxB + 0.1625 \times xAxC - 0.1875 \times xBxC - 0.2375 \times xA^2 + 0.0577 \times xB^2 - 0.1423 \times xC^2 \quad (6)$$

The adjusted R squared was the lowest (0.909) among the analyzed responses. The lack of fit was not significant (p-value: 0.1212). No parameters exerted a statistically significant influence when considered individually. The only regression coefficient resulting in significance was the interaction effect of germination time and temperature. There was correspondence between the observed and predicted minimum and maximum values for the same combination of parameters. The resulting response surfaces can be defined as a saddle point or min-max, thus an optimum area could not be identified. Figure 4 illustrates the pattern of the response as time and temperature change when the degree of steeping is kept constant at 40 g/100 g and 46 g/100 g. Three different situations were identified by setting the degree of steeping at the three different

levels defined by the experimental design. AAL values above 74.0% could be detected for short germination times and high temperatures when the degree of steeping was held at 40 g/100 g (Figure 4, subplot a). In particular, as the degree of steeping increased from the lowest to the highest level, the response surface tended to shift within lower boundaries defined by the predicted AAL. Areas of increasing response can be observed both at 4 days and 18 degrees and at 6 days and 12 degrees when humidity is maintained at the highest level (Figure 4, subplot b). Generally, the minimum response tended to be located at the corner of the experimental region, at the lowest and highest combination levels for germination time and temperature.

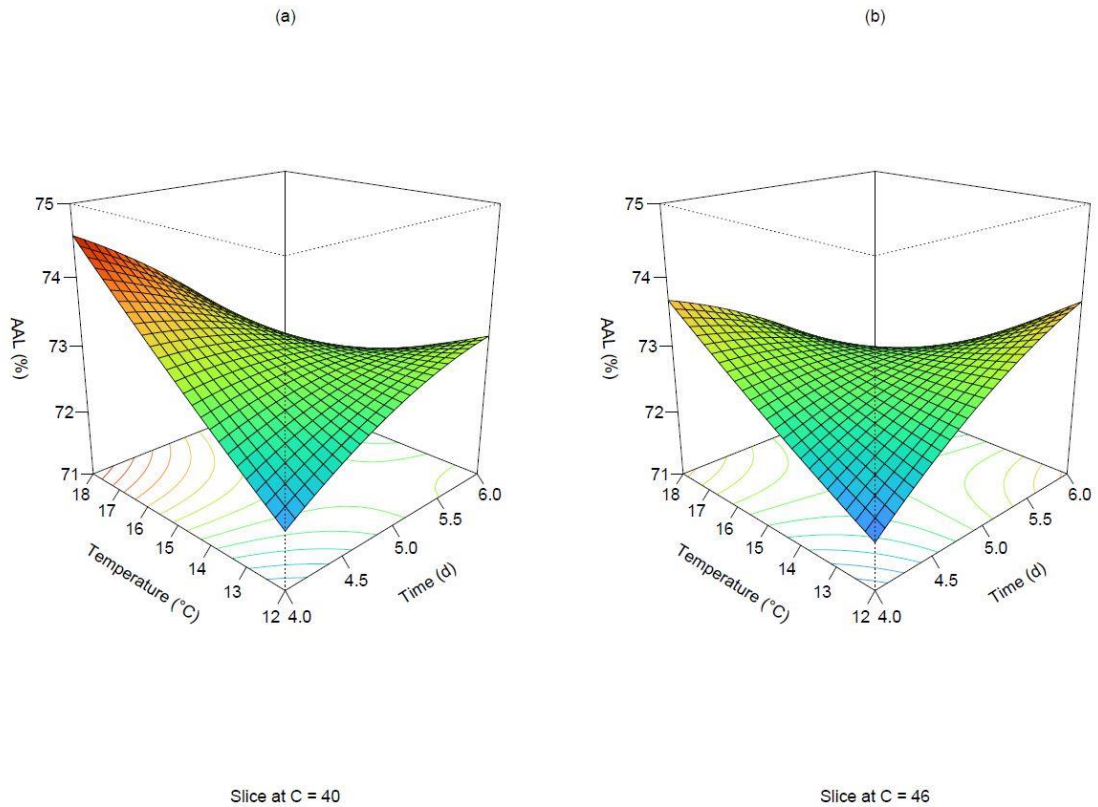


Figure 4. Surface plots of the predicted apparent attenuation limit. The degree of steeping is held at 40 g/100 g (a) and 46 g/100 g (b).

3.5. Viscosity

Viscosity can be measured in both Congress and isothermal (65 °C) wort. Both methods provide information on the cytolysis modification of the malt. In particular, the latter gives an insight into the differences between different malts [40]. Recommended values for barley malt are below 1.56 and 1.60 mPa × s for viscosity measured in Congress and isothermal wort, respectively [54]. Highly modified malts exhibit lower viscosity levels [57]. Unconventional malts other than barley may lead to lautering problems due to the increased wort viscosity [58]. In order to ensure its reduction to levels that allow for good filtration, enzyme activity must be promoted by appropriate timing and temperatures during brewing. Indeed, as amylolytic enzyme efficiency decreases as a result of an excessive shift from their temperature optimum, viscosity tends to rise [59]. The lowest viscosity levels were obtained in malts with extracts close to or above 83%. The ranges of observed values were slightly higher for the viscosity measured in the isothermal mash (5.42–8.93 mPa × s) than in the Congress mash (from 4.19 to 7.89 mPa × s). Both responses were described by a quadratic model, and their equations are reported as follows:

$$\begin{aligned} \text{Viscosity} = & 4.7654 - 0.7291 \times xA - 0.9050 \times xB - 0.1350 \times xC + 0.3675 \times xAxB + 0.0150 \times xAxC \\ & + 0.1100 \times xBxC + 0.2929 \times xA^2 + 0.4933 \times xB^2 \end{aligned} \quad (7)$$

$$\text{Viscosity } 65^\circ\text{C} = 6.4582 - 0.6624 \times xA - 0.8020 \times xB - 0.3590 \times xC - 0.0375 \times xAxB - 0.0500 \times xAxC + 0.1675 \times xBxC + 0.6258 \times xB^2 \quad (8)$$

The adjusted R squared for both models was higher than 0.93, and the lack of fit was non-significant (p-values: 0.0825167 and 0.2749261 for viscosity measured in Congress and isothermal wort). All linear terms, the interaction and the quadratic terms for germination time and temperature resulted in significance for the viscosity measured in the Congress wort. In particular, both factors exerted a greater negative influence. A negative effect was also exerted by the degree of steeping, but to a lesser extent. In both scenarios, it can be concluded that as time, temperature and degree of steeping increase, a consequent drop in viscosity is expected. Under these conditions, a gradual cytolytic modification of the cell wall components affecting the wort viscosity occurs. Figure 5 shows the interactive effect of time and temperature when the degree of steeping is held at a constant level of 46 g/100 g for both predicted responses. As depicted in the two subplots, the response surfaces turned into a concave shape. Prolonging the germination time at increasing temperatures resulted in a decrease in the predicted responses. Particularly, a plateau can be observed below 15 °C and after 5 days of germination, at which the lowest viscosity values were predicted by the two models.

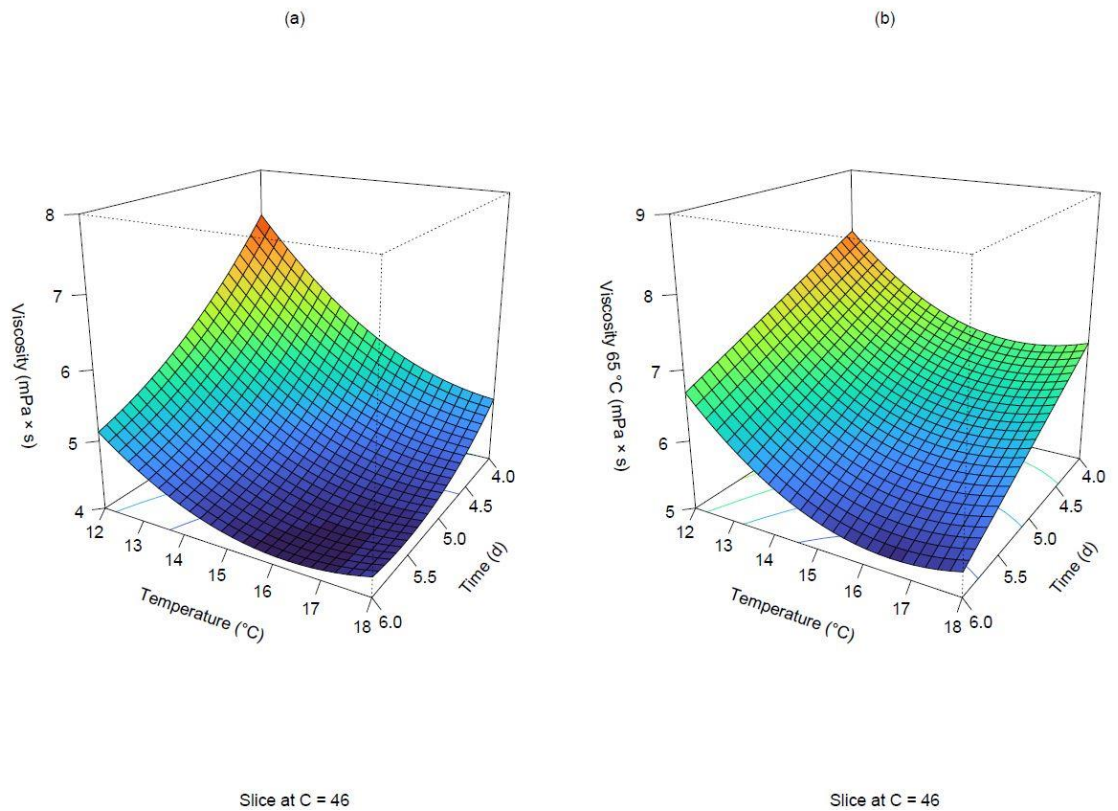


Figure 5. Surface plots of viscosities. Predicted viscosities in Congress (a) and isothermal mash (b) when the degree of steeping is held at 46 g/100 g.

3.6. Multicriteria Optimization

The optimization process was performed by means of the following two different methods: the desirability function approach and the genetic algorithm. The target was to maximize extract and ALL, while keeping the KI and the viscosity measured in Congress and the isothermal wort as low as possible. Therefore, the function was set up in view of the validity of the fitted models and in such a way that all the following three events occurring during malting are accounted for: amylolysis, proteolysis and cytolysis. KI was considered an indicator of proteolysis. A balance between soluble nitrogen and protein content is generally sought, so a KI within a certain range

is preferable. High values may adversely affect certain beer characteristics. For instance, foam stability in beer was found to be negatively correlated to KI, although with different magnitudes [60,61]. Overall, the measured KI values were remarkably high (43.3–62.0%) compared to the reference values of barley and wheat malt. Taking this into account, when setting the individual desirability function for KI, the aim was to minimize it.

3.6.1. Desirability Function

The acceptability boundaries for the responses were set on the basis of the minimum and maximum values of the experimental results. Each response was transformed into an individual desirability value ranging from 0 to 1. A larger-is-better function was applied to maximize extract and AAL. A smaller-is-better function was used to minimize KI and viscosity measured in Congress and isothermal wort. The scaling factor for both maximization and minimization was set at 1. A data frame containing combinations of parameters in coded and uncoded units was created. Based on the fitted models, the selected responses to be optimized were predicted for each combination of independent variables. Hence, individual desirability values were calculated and finally combined to obtain the overall desirability D. Two areas of feasible solutions were identified, as depicted in Figure 6.

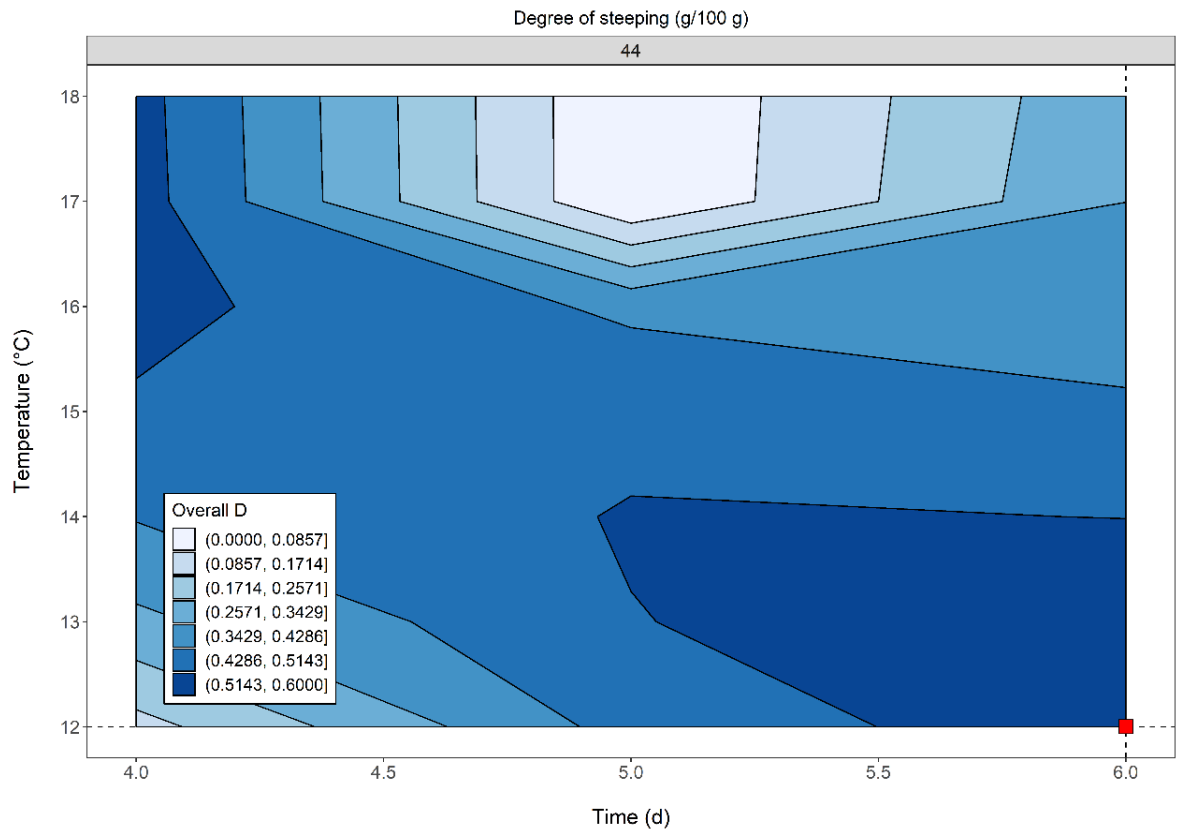


Figure 6. Contour plot of the overall desirability D. Color shades refer to specific D ranges: from light to dark blue, D increases. The dotted lines meet at the highest computed overall desirability value D (0.564), denoted by the red.

Indeed, maximum desirability values were obtained for the same degree of steeping but at opposite levels for the temperature and time parameters. Thus, at a fixed degree of steeping of 44%, satisfactory responses meeting the above criteria can be obtained by combining long germination times at low temperatures, or alternatively for shorter germination periods at higher temperatures. The highest value computed by the function was 0.568 at 6 days, 12 °C and 44 g/100 g. The following optimized responses were predicted for this combination of parameters: extract 82.7% d.m.; KI 55.6%; AAL 73.6%; viscosity 5.27 mPa × s; viscosity (65 °C) 7.07 mPa × s.

3.6.2. Genetic Algorithm

A genetic algorithm was applied to find a global optimum implementing the desirability function specified above as the fitness function. Specific information on the definition and typologies of genetic operators can be found in the 'GA' package [35]. In the case of optimization problems, the type of genetic algorithm is defined as 'real-valued'; the minimum and maximum limits of the search space were defined within the boundaries of the experimental design; the population function was defined as 'gareal_population', which generates a random and uniform population of real values within the constraints of the search space; selection was defined as 'gareal_lsSelection'; the genetic operator crossover was chosen as 'gareal_laCrossover' which stands for local arithmetic crossover; mutation was defined as "gareal_raMutation" which stands for random and uniform mutation; the elitism operator, equal to the number of individuals with the best fitness surviving each generation or iteration, was set to 5%; the number of individuals within the population was set to 100; the maximum number of iterations was equal to 100; the probability of crossover was set at 0.8; the probability of a mutation occurring within a parent chromosome was set at 0.1; a specific seed was set to generate reproducible results. Each chromosome consisted of combinations of the three independent variables. For each specific combination of parameters within a chromosome, a predicted response was obtained, the fit of which was assessed by means of the fitness function. The worst solution had an overall desirability of 0.4081. After the 7th iteration, the best solution of 0.5709 was identified. Thereafter, no improvement was detected during subsequent iterations (Figure 7).

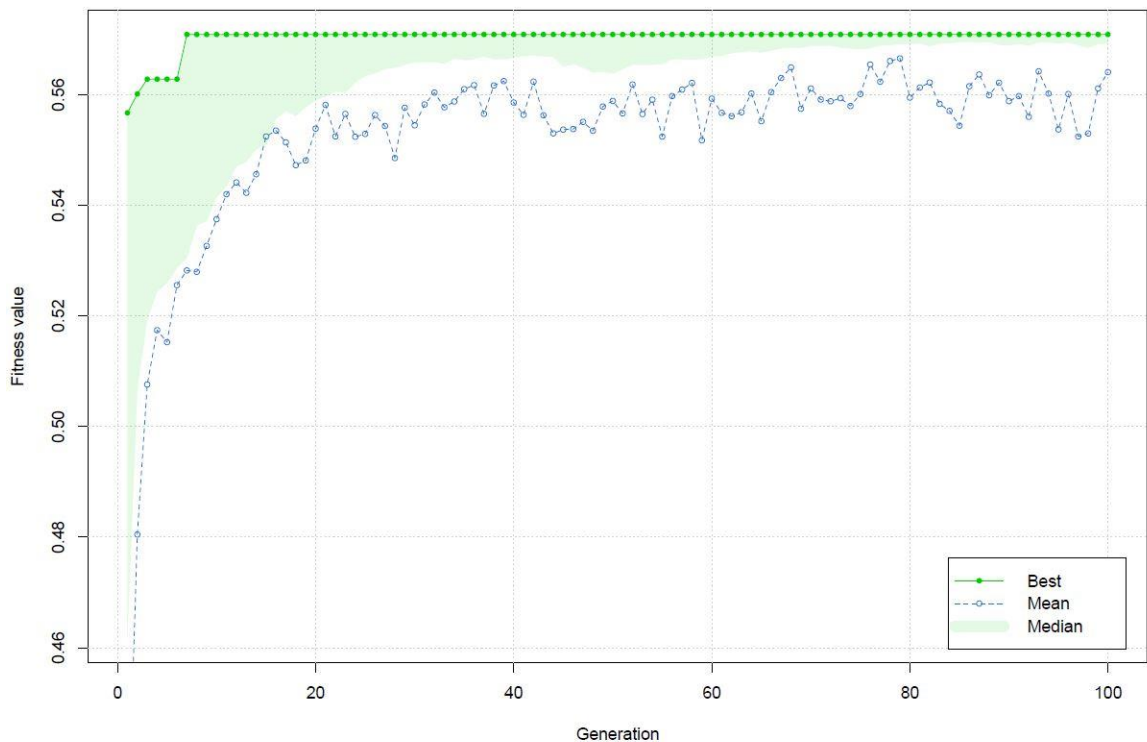


Figure 7. Plot of best, mean and median fitness values recorded during the iterations of the genetic algorithm. The fitness value represents the overall desirability.

The best solution found by the genetic algorithm was given in coded units ($x_A = 1$, $x_B = -0.8352368$, $x_C = 0.06473789$). For this reason, the equations defining the relationship between coded and uncoded parameters (Equations (1)–(3)) were used to calculate the real values. The combination of parameters optimizing the overall D was the following: 6 d, 12.5 °C and 43.2 g/100 g. The regression equations in uncoded units for all models were used to compute the predicted

values, taking into account the best combination of predicted parameters as follows: extract 82.9%; KI 56.0%; AAL 73.4%; viscosity 5.11 mPa × s; viscosity (65 °C) 6.89 mPa × s. These results coincided with those predicted by the desirability function for the same parameter combination, however, for a lower total desirability value.

4. Conclusions

The adopted experimental design made it possible to discern how malting parameters and their levels can influence the quality characteristics of rye malt. The entire response surface analysis and subsequent optimization process were carried out on Rstudio, the R-integrated development environment, showing how the use of open-source software can prove effective as an alternative to expensive software. Analysis of the response surfaces revealed that germination time and temperature strongly influenced the modification of rye malt in terms of amylolytic, proteolytic and cytolytic activity. The influence of the degree of steeping, on the whole, was less pronounced. The desirability function and the genetic algorithm gave similar results. The first method allowed us to navigate a broader space of possible combinations. Nevertheless, the overall desirability D calculated by the genetic algorithm was slightly higher. Both optimization techniques provided comparable results. Hence, the combination of the two optimization techniques proved to be appropriate as part of the surface response methodology. It is interesting to note that two optimal situations can potentially be used to achieve the desired goals at the same degree of steeping of 44 g/100 g. Malting for only 4 days of germination at high temperatures would significantly reduce the costs associated with the process. Nevertheless, the combination predicted at 6 days and 12 °C would reduce the KI to a greater extent. The cereal under study was characterized by a high protein content, so aiming for the best situation with regard to proteolytic activity was one of the objectives. For this reason, the suggested malting parameter combination for this rye landrace is as follows: 6 days at 12 °C and 44 g/100 g. Further studies would be needed to fully confirm these results and set up new tests by exploring a different experimental region on the basis of the developed models. Nonetheless, the study helped to give a greater understanding of the amylolytic, cytolytic and proteolytic modifications of rye during malting, the information of which is significantly narrower when compared to that of barley and wheat for malting purposes.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/foods11223561/s1>, Table S1. Comparison of model performance indices; Figure S1. Plot of externally studentized residuals vs. predicted responses. Each subplot refers to a specific quality attribute: (a) extract; (b) KI, Kolbach index; (c) AAL, apparent attenuation limit; (d) viscosity in the Congress wort; (e) viscosity in the isothermal mash; Figure S2. Plot of externally studentized residuals vs. run number. Each subplot refers to a specific quality attribute: (a) extract; (b) KI, Kolbach index; (c) AAL, apparent attenuation limit; (d) viscosity in the Congress wort; (e) viscosity in the isothermal mash; Figure S3. Plot of predicted vs. actual responses. Each subplot refers to a specific quality attribute: (a) extract; (b) KI, Kolbach index; (c) AAL, apparent attenuation limit; (d) viscosity in the Congress wort; (e) viscosity in the isothermal mash.

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References

1. Garavaglia, C. Industry Evolution: Evidence from the Italian Brewing Industry. *Compet. Chang.* **2022**, *26*, 75–95. [[CrossRef](#)]
2. Cipollaro, M.; Fabbrizzi, S.; Sottini, V.A.; Fabbri, B.; Menghini, S. Linking Sustainability, Embeddedness and Marketing Strategies: A Study on the Craft Beer Sector in Italy. *Sustainability* **2021**, *13*, 10903. [[CrossRef](#)]
3. Holtkamp, C.; Shelton, T.; Daly, G.; Hiner, C.C.; Hagelman, R.R., III. Assessing Neolocalism in Microbreweries. *Pap. Appl. Geogr.* **2016**, *2*, 66–78. [[CrossRef](#)]
4. Colombo, F.; Franguelli, N.; Licheri, G.; Ghidoli, M.; Cassani, E.; Castelli, L.; Pasquali, M.; Bresciani, A.; Marti, A.; Dell'Anno, M.; et al. Agriculture in Marginal Areas: Reintroduction of Rye and Wheat Varieties for Breadmaking in the Antrona Valley. *Agronomy* **2022**, *12*, 1695. [[CrossRef](#)]
5. Zeven, A. Landraces: A Review of Definitions and Classifications. *Euphytica* **1998**, *104*, 127–139. [[CrossRef](#)]
6. ARSAC. Available online: <https://www.arsacweb.it/la-segale-il-cereale-da-rilanciare-nei-parchi-nazionali-calabresi/> (accessed on 29 August 2022).
7. Korzun, V.; Ponomareva, M.L.; Sorrells, M.E. Economic and Academic Importance of Rye. In *The Rye Genome*; Rabanus-Wallace, M.T., Stein, N., Eds.; Springer International: Cham, Switzerland, 2021; pp. 1–12, ISBN 978-3-030-83383-1.
8. Behre, K.E. The history of rye cultivation in Europe. *Veg. Hist. Archaeobotany* **1992**, *1*, 141–156. [[CrossRef](#)]
9. Schreiber, M.; Özkan, H.; Komatsuda, T.; Mascher, M. Evolution and Domestication of Rye. In *The Rye Genome*; Rabanus-Wallace, M.T., Stein, N., Eds.; Springer International: Cham, Switzerland, 2021; pp. 85–100, ISBN 978-3-030-83383-1.
10. Németh, R.; Tömösközi, S. Rye: Current State and Future Trends in Research and Applications. *Acta Aliment.* **2021**, *50*, 620–640. [[CrossRef](#)]
11. Wrigley, C.; Bushuk, W. Rye: Grain-Quality Characteristics and Management of Quality Requirements. In *Cereal Grains*, 2nd ed.; Chapter 7; Wrigley, C., Batey, I., Miskelly, D., Eds.; Woodhead Publishing: Sawston, UK, 2017; pp. 153–178, ISBN 978-0-08-100719-8.
12. Food and Agricultural Organization of United Nations (FAO). FAOSTAT. Available online: <https://www.fao.org/faostat/en/#data/QCL> (accessed on 24 August 2022).
13. Kaur, P.; Sandhu, K.S.; Purewal, S.S.; Kaur, M.; Singh, S.K. Rye: A Wonder Crop with Industrially Important Macromolecules and Health Benefits. *Food Res. Int.* **2021**, *150*, 110769. [[CrossRef](#)]

14. Jonsson, K.; Andersson, R.; Knudsen, K.E.B.; Hallmans, G.; Hanhineva, K.; Katina, K.; Kolehmainen, M.; Kyrø, C.; Langton, M.; Nordlund, E.; et al. Rye and Health—Where Do We Stand and Where Do We Go? *Trends Food Sci. Technol.* **2018**, *79*, 78–87. [[CrossRef](#)]
15. Kulichová, K.; Sokol, J.; Nemeček, P.; Maliarová, M.; Maliar, T.; Havrlentová, M.; Kraic, J. Phenolic Compounds and Biological Activities of Rye (*Secale Cereale* L.) Grains. *Open Chem.* **2019**, *17*, 988–999. [[CrossRef](#)]
16. Vuholm, S.; Nielsen, D.S.; Iversen, K.N.; Suhr, J.; Westermann, P.; Krych, L.; Andersen, J.R.; Kristensen, M. Whole-Grain Rye and Wheat Affect Some Markers of Gut Health without Altering the Fecal Microbiota in Healthy Overweight Adults: A 6-Week Randomized Trial. *J. Nutr.* **2017**, *147*, 2067–2075. [[CrossRef](#)]
17. Adhikari, K.B.; Tanwir, F.; Gregersen, P.L.; Steffensen, S.K.; Jensen, B.M.; Poulsen, L.K.; Nielsen, C.H.; Høyer, S.; Borre, M.; Fomsgaard, I.S. Benzoxazinoids: Cereal Phytochemicals with Putative Therapeutic and Health-Protecting Properties. *Mol. Nutr. Food Res.* **2015**, *59*, 1324–1338. [[CrossRef](#)]
18. Pihlava, J.-M.; Kurtelius, T. Determination of Benzoxazinoids in Wheat and Rye Beers by HPLC-DAD and UPLC-QTOF MS. *Food Chem.* **2016**, *204*, 400–408. [[CrossRef](#)]
19. Stika, H.-P. Early Iron Age and Late Mediaeval Malt Finds from Germany—Attempts at Reconstruction of Early Celtic Brewing and the Taste of Celtic Beer. *Archaeol. Anthropol. Sci.* **2011**, *3*, 41–48. [[CrossRef](#)]
20. Pomeranz, Y.; Standridge, N.N.; Schreck, J.J.; Goplin, E.D. Rye in Malting and Brewing 1. *Crop Sci.* **1973**, *13*, 213–215. [[CrossRef](#)]
21. Walker, G.; Hill, A. *Saccharomyces Cerevisiae* in the Production of Whisk(e)y. *Beverages* **2016**, *2*, 38. [[CrossRef](#)]
22. Bogdan, P.; Kordialik-Bogacka, E. Alternatives to Malt in Brewing. *Trends Food Sci. Technol.* **2017**, *65*, 1–9. [[CrossRef](#)]
23. Marsh, A.J.; Hill, C.; Ross, R.P.; Cotter, P.D. Fermented Beverages with Health-Promoting Potential: Past and Future Perspectives. *Trends Food Sci. Technol.* **2014**, *38*, 113–124. [[CrossRef](#)]
24. Ekberg, J.; Gibson, B.; Joensuu, J.J.; Krogerus, K.; Magalhães, F.; Mikkelsen, A.; Seppänen-Laakso, T.; Wilpola, A. Physicochemical Characterization of Sahti, an ‘Ancient’ Beer Style Indigenous to Finland: Physicochemical Characterization of Sahti. *J. Inst. Brew.* **2015**, *121*, 464–473. [[CrossRef](#)]
25. Segobia Muñoz, S.A. Evaluation of the Addition of Rye (*Secale cereale*) in the Formulation of Belgian Pale Ale Craft Beer. *Enfoque UTE* **2022**, *13*, 14–28. [[CrossRef](#)]
26. BJCP. Available online: <https://www.bjcp.org/bjcp-style-guidelines/> (accessed on 29 August 2022).
27. Hallam, N.D. Embryogenesis and germination in rye (*Secale cereale* L.). *Planta* **1972**, *104*, 157–166. [[CrossRef](#)]
28. Briggs, D.E. *Malts and Malting*, 1st ed.; An Imprint of Thomson Science; Blackie Academic & Professional: London, UK, 1998.
29. Myers, R.H.; Montgomery, D.C.; Anderson-Cook, C.M. *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*, 3rd ed.; JohnWiley & Sons: New York, NY, USA, 2009.
30. Khuri, A.I.; Mukhopadhyay, S. Response Surface Methodology. *WIREs Comput. Stat.* **2010**, *2*, 128–149. [[CrossRef](#)]
31. Kuhn, M. R Package, Version 2.1; Desirability: Function Optimization and Ranking via Desirability Functions; R Foundation for Statistical Computing: Vienna, Austria, 2016. Available online: <https://CRAN.R-project.org/package=desirability> (accessed on 23 May 2022).
32. Holland, J.H. Genetic Algorithms. *Sci. Am.* **1992**, *267*, 66–73. Available online: <http://www.jstor.org/stable/24939139> (accessed on 23 May 2022). [[CrossRef](#)]
33. Álvarez, M.J.; Ilzarbe, L.; Viles, E.; Tanco, M. The Use of Genetic Algorithms in Response Surface Methodology. *Qual. Technol. Quant. Manag.* **2009**, *6*, 295–307. [[CrossRef](#)]

34. Thongsook, S. Using the GA Package in R Program and Desirability Function to Develop a Multiple Response Optimization Procedure in Case of Two Responses. *Thail. Stat. Jan.* **2018**, 16, 64–76. Available online: <http://statassoc.or.th> (accessed on 23 May 2022).
35. Scrucca, L. GA: A Package for Genetic Algorithms in R. *J. Stat. Soft.* **2013**, 53, 1–37. [[CrossRef](#)]
36. Goldberg, D.E. Genetic Algorithms. In *Search, Optimization and Machine Learning*, 1st ed.; Addison-Wesley Longman Publishing Co., Inc.: Boston, MA, USA, 1989; ISBN 0-201-15767-5.
37. Chawanda, E.T.; Manhokwe, S.; Jombo, T.Z.; Mugadza, D.T.; Njini, M.; Manjeru, P. Optimisation of Malting Parameters for Quinoa and Barley: Application of Response Surface Methodology. *J. Food Qual.* **2022**, 2022, 5279177. [[CrossRef](#)]
38. Muñoz-Insa, A.; Gastl, M.; Zarnkow, M.; Becker, T. Optimization of the Malting Process of Oat (*Avena sativa* L.) as a Raw Material for Fermented Beverages. *Span. J. Agric. Res.* **2011**, 9, 510–523. [[CrossRef](#)]
39. Muñoz-Insa, A.; Selciano, H.; Zarnkow, M.; Becker, T.; Gastl, M. Malting Process Optimization of Spelt (*Triticum spelta* L.) for the Brewing Process. *LWT-Food Sci. Technol.* **2013**, 50, 99–109. [[CrossRef](#)]
40. Methner, F.-J. Raw Materials: Adjuncts, Barley, Malt, Hops and Hop Products: Collection of Brewing Analysis Methods of the Mitteleuropäische Brautechnische Analysenkommission; MEBAK®: Freising, Germany, 2018.
41. FAO. Available online: <https://www.fao.org/3/y5022e/y5022e03.htm#:~:text=On%20the%20basis%20of%20early,nitrog en%20content%20into%20protein%20content> (accessed on 28 September 2022).
42. Lenth, R.V. Response-Surface Methods in R, Using Rsm. *J. Stat. Soft.* **2009**, 32, 1–17. [[CrossRef](#)]
43. R Core Team. R: A Language and Environment for Statistical Computing (R Version 4.1.0); R Foundation for Statistical Computing: Vienna, Austria, 2021. Available online: <https://www.R-project.org/> (accessed on 2 March 2022).
44. Lüdecke, D.; Ben-Shachar, M.; Patil, I.; Waggoner, P.; Makowski, D. Performance: An R Package for Assessment, Comparison and Testing of Statistical Models. *J. Open Source Softw.* **2021**, 6, 3139. [[CrossRef](#)]
45. Venables, W.N.; Ripley, B.D. *Modern Applied Statistics with S*, 4th ed.; Springer: New York, NY, USA, 2022; ISBN 0-387-95457-0. Available online: <https://www.stats.ox.ac.uk/pub/MASS4/> (accessed on 12 April 2022).
46. Wickham, H. *Ggplot2: Elegant Graphics for Data Analysis*; Springer: New York, NY, USA, 2016; ISBN 978-3-319-24277-4. Available online: <https://ggplot2.tidyverse.org> (accessed on 12 April 2022).
47. Garnier, S.; Ross, N.; Rudis, R.; Camargo, A.P.; Sciaini, M.; Scherer, C. R Package, Version 0.6.2; Rvision—Colorblind-Friendly Color Maps for R; R Foundation for Statistical Computing: Vienna, Austria, 2021. Available online: <https://sjmgarnier.github.io/viridis/> (accessed on 13 April 2022).
48. Pedersen, T.L. R Package, Version 1.1.1; Patchwork: The Composer of Plots; R Foundation for Statistical Computing: Vienna, Austria, 2020. Available online: <https://CRAN.R-project.org/package=patchwork> (accessed on 12 April 2022).
49. Humberg, S.; Grund, S. Response Surface Analysis with Missing Data. *Multivar. Behav. Res.* **2022**, 57, 581–602. [[CrossRef](#)]
50. van Buuren, S.; Groothuis-Oudshoorn, K. Mice: Multivariate Imputation by Chained Equations in R. *J. Stat. Softw.* **2011**, 45, 1–67. [[CrossRef](#)]
51. Borkowski, J.J. Center Points. In *Encyclopedia of Statistics in Quality and Reliability*; Ruggeri, F., Kenett, R.S., Faltin, F.W., Eds.; John Wiley & Sons, Ltd: Chichester, UK, 2008. [[CrossRef](#)]
52. Cabras, P.; Tuberoso, C.I.G. *Analisi dei Prodotti Alimentari*; Piccin Nuova Libreria S.p.A.: Padova, Italy, 2014.
53. Wang, Y.; Jin, Z.; Barr, J.; Gillespie, J.; Simsek, S.; Horsley, R.; Schwarz, P. Micro-Malting for the Quality Evaluation of Rye (*Secale Cereale*) Genotypes. *Fermentation* **2018**, 4, 50. [[CrossRef](#)]

54. Back, W.; Gastl, M.; Krottenthaler, M.; Narziss, L.; Zarnkow, M. *Brewing Techniques in Practice: An In-Depth Guide with Problem Solving Strategies*; Hans Carl Fachverlag: Nürnberg, Germany, 2019; Volume 1, p. 26.
55. Hübner, F.; Arendt, E.K. Comparison of Protein Degradation as a Consequence of Germination Time and Temperature in Rye and Barley Malts. *J. Am. Soc. Brew. Chem.* **2010**, *68*, 195–203. [[CrossRef](#)]
56. Narziß, L. *Abriss der Bierbrauerei*, 7th ed. Wiley-VCH: Weinheim, Germany, 2005.
57. Gastl, M.; Kupetz, M.; Becker, T. Determination of Cytolytic Malt Modification—Part II: Impact on Wort Separation. *J. Am. Soc. Brew. Chem.* **2021**, *79*, 66–74. [[CrossRef](#)]
58. Blšáková, L.; Gregor, T.; Mešťánek, M.; Hřivna, L.; Kumbár, V. The Use of Unconventional Malts in Beer Production and Their Effect on the Wort Viscosity. *Foods* **2021**, *11*, 31. [[CrossRef](#)]
59. Laus, A.; Endres, F.; Hutzler, M.; Zarnkow, M.; Jacob, F. Isothermal Mashing of Barley Malt: New Insights into Wort Composition and Enzyme Temperature Ranges. *Food Bioprocess Technol.* **2022**, *15*, 2294–2312. [[CrossRef](#)]
60. Evans, D.E.; Sheehan, M.C.; Stewart, D.C. The Impact of Malt Derived Proteins on Beer Foam Quality. Part II: The Influence of Malt Foam-Positive Proteins and Non-Starch Polysaccharides on Beer Foam Quality. *J. Inst. Brew.* **1999**, *105*, 171–178. [[CrossRef](#)]
61. Kordialik-Bogacka, E.; Antczak, N. Prediction of Beer Foam Stability from Malt Components. *Czech J. Food Sci.* **2011**, *29*, 243–249. [[CrossRef](#)]

Supplementary Materials

Table S1. Comparison of model performance indices.

Response	Model	AIC ¹	BIC ²	R squared	Adjusted R squared	RMSE ³	Sigma	Performance score
Extract	FO ⁶	45.923	50.089	0.564	0.463	0.696	0.796	0.00%
	TWI ⁷	39.922	46.588	0.785	0.655	0.489	0.638	27.48%
	PQ ⁸	11.246	20.411	0.972	0.936	0.176	0.275	82.04%
	CM ⁹	10.747	16.579	0.956	0.937	0.220	0.273	97.97%
KI ⁴	FO	94.356	98.522	0.730	0.667	2.255	3.308	0.00000122%
	TWI	91.893	98.559	0.836	0.737	2.255	2.940	19.63%
	PQ	64.836	74.001	0.976	0.946	0.853	1.329	97.50%
	CM	67.011	73.676	0.962	0.939	1.085	1.414	84.93%
AAL ⁵	FO	49.119	53.285	0.065	-0.151	0.765	0.874	0.00%
	TWI	13.014	19.679	0.921	0.874	0.222	0.289	67.41%
	PQ	7.551	16.716	0.960	0.908	0.158	0.247	100.00%
	CM	13.014	19.679	0.921	0.874	0.222	0.289	67.41%
Viscosity	FO	31.257	35.423	0.788	0.739	0.452	0.517	0.000000289%
	TWI	30.213	36.878	0.860	0.776	0.368	0.479	14.02%
	PQ	-10.507	-1.342	0.991	0.979	0.093	0.145	79.75%
	CM	-11.937	-3.605	0.991	0.981	0.095	0.138	99.90%
Viscosity 65 °C	FO	24.538	28.705	0.827	0.787	0.371	0.424	5.49%
	TWI	28.572	35.238	0.846	0.753	0.350	0.457	3.86%
	PQ	7.522	16.687	0.969	0.928	0.158	0.246	75.49%
	CM	5.880	13.379	0.964	0.936	0.169	0.233	98.56%

¹ AIC, Akaike information criterion; ² BIC, Bayesian information criterion; ³ RMSE, Root Mean Square Error; ⁴ KI, Kolbach index; ⁵ AAL, apparent attenuation limit; ⁶ FO, first order; ⁷ TWI, two-way interaction; ⁸ PQ, pure quadratic; ⁹ CM, customized model (excluding some non-significant terms while maintaining hierarchy) The selected models have been reported in bold.

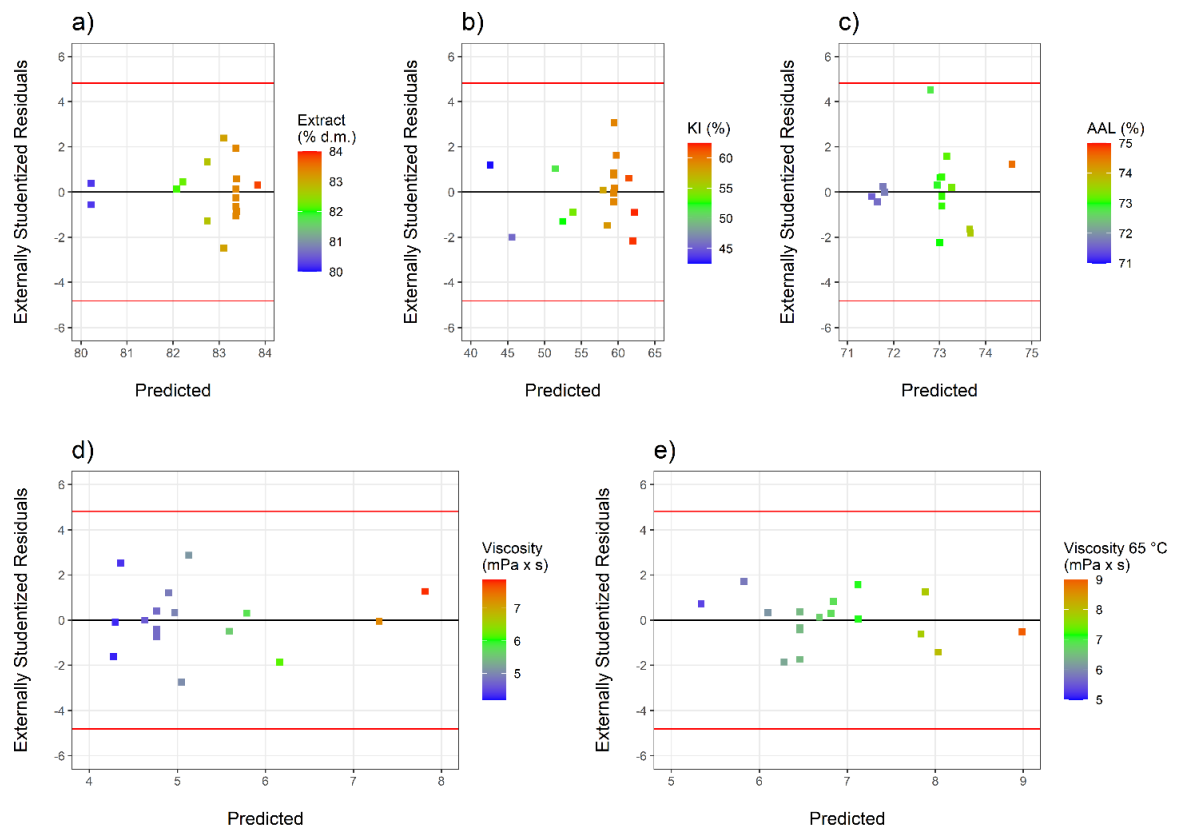


Figure S1. Plot of externally studentized residuals vs predicted responses. Each subplot refers to a specific quality attribute: a) extract; b) KI, Kolbach index; c) AAL, apparent attenuation limit; d) viscosity in the Congress wort; e) viscosity in the isothermal mash.

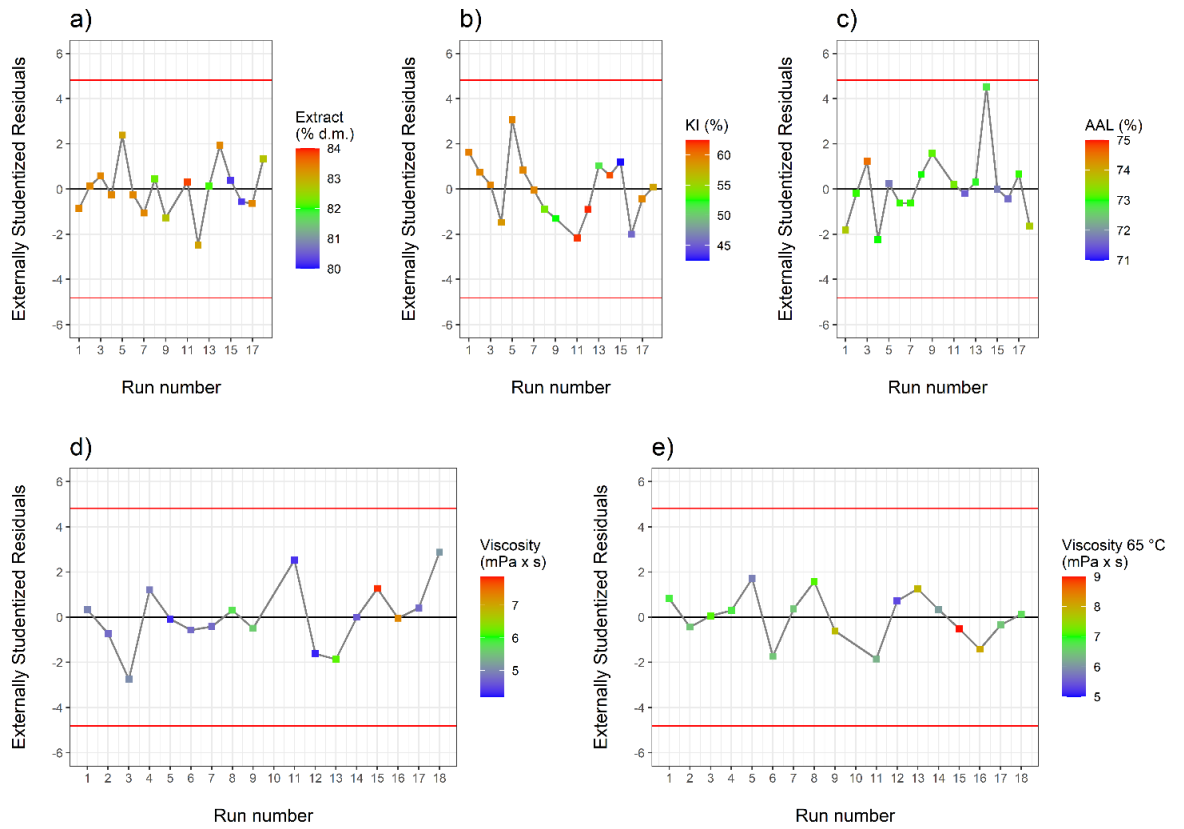


Figure S2. Plot of externally studentized residuals vs run number. Each subplot refers to a specific quality attribute: a) extract; b) KI, Kolbach index; c) AAL, apparent attenuation limit; d) viscosity in the Congress wort; e) viscosity in the isothermal mash.

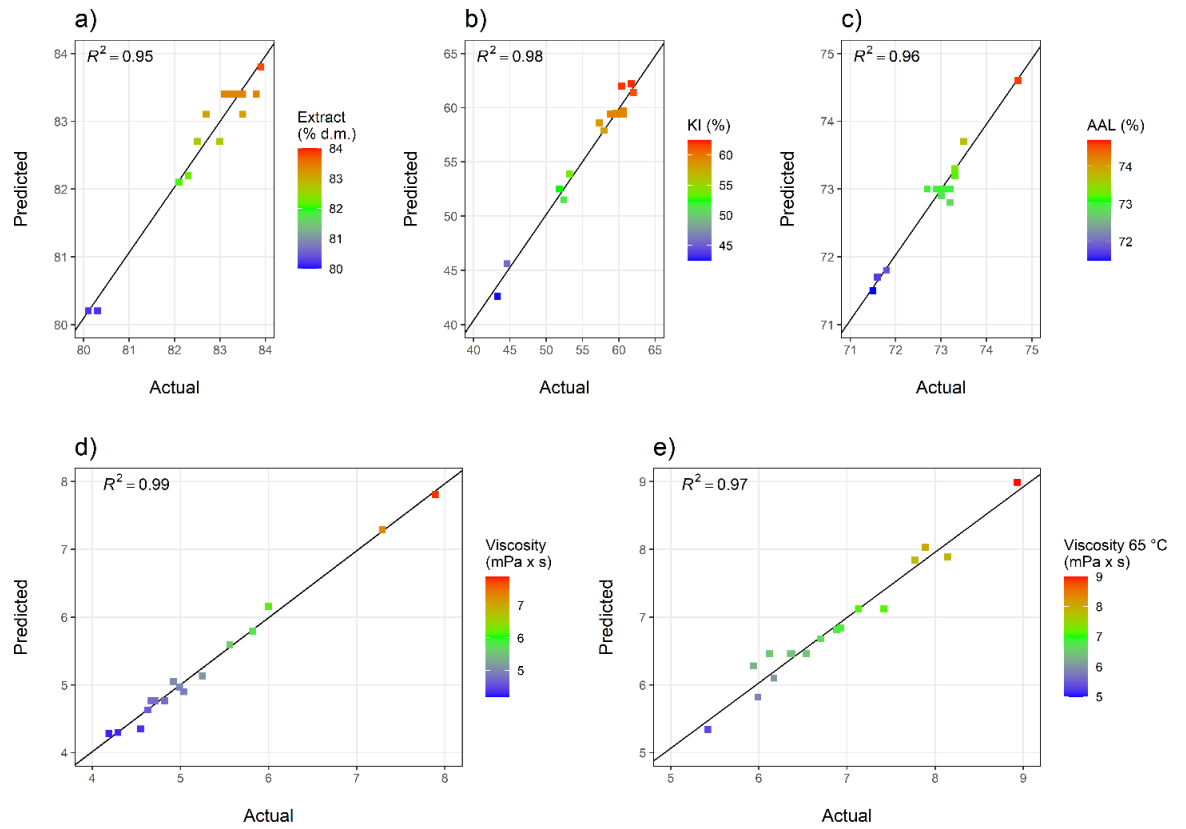


Figure S3. Plot of predicted vs actual responses. Each subplot refers to a specific quality attribute: a) extract; b) KI, Kolbach index; c) AAL, apparent attenuation limit; d) viscosity in the Congress wort; e) viscosity in the isothermal mash.

Capitolo 6

Conclusions and Future Perspectives

The thesis work done during the three years of doctoral studies focused on optimizing the malting of a wheat landrace and a rye landrace. These and other local landraces of cereals are grown in some areas of the Calabria region, the grain of which is commonly used for other food processing. Given the presence of numerous craft breweries in the region and the concomitant absence of local raw materials to be used in brewing (normally utilized in unmalted form), the focus was precisely on specific varieties that presented a link to the territory in which these activities operate. The thesis was divided into three parts, starting with an agronomic assessment, which was relevant for understanding the relationship between the performance of the chosen varieties and the area of cultivation, in order to obtain preliminary information to set up further investigations. After two years of agronomic trials in the same area, it was possible to conclude that the interaction effect between genotypes and trial year was significant and resulted in a decrease for most of the bioagronomic traits evaluated. As expected, malting barley showed excellent agronomic performance, confirming that quality production for the malting industry is achievable in southern Italy. Compared with the results obtained with barley, the landraces were characterized by high plant heights, higher hectoliter weights and longer earing periods. At the same time, grain yields were lower than those of barley, but the discrepancy between the two growing seasons was greater for the latter species, confirming a greater ability of the old populations to mitigate environmental effects. Further studies on agronomic management specific to landraces are necessary in view of a possible malting application. The characteristics of landraces were obviously inferior, as they are not varieties commonly intended for malting. Some differences were found between wheat and rye: wheat malts were characterized by low viscosity and low β -glucan content if compared with rye. Results showed that all the rye landraces presented similar malting qualities. After these initial field and analytical evaluations, and after then identifying two wheat and rye landraces among those under study, the work conducted in Germany was focused on optimizing their malting to increase specific quality attributes. This study was carried out using the surface response methodology. The optimization of a product in which several variables contribute to defining its quality is not simple. Only those models that showed adequacy were chosen and used to find the optimum of process parameters. Through a desirability function and a genetic algorithm, it was possible to predict the combination of time, temperature and humidity, which may allow increasing amylolytic, cytolitic and proteolytic modifications of the chosen grains. This work thus allowed for a deeper and more specific understanding of how different settings affect grain transformation during malting. Further studies will be needed in the future to verify these predictions, as well as to evaluate the use of the malts thus optimized in brewing tests.