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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.

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

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

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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 evaluted 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 vegetation 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 in 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 (Narziβ & 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.

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

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 2^3 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.

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

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)	AAL (%)
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.

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.

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.

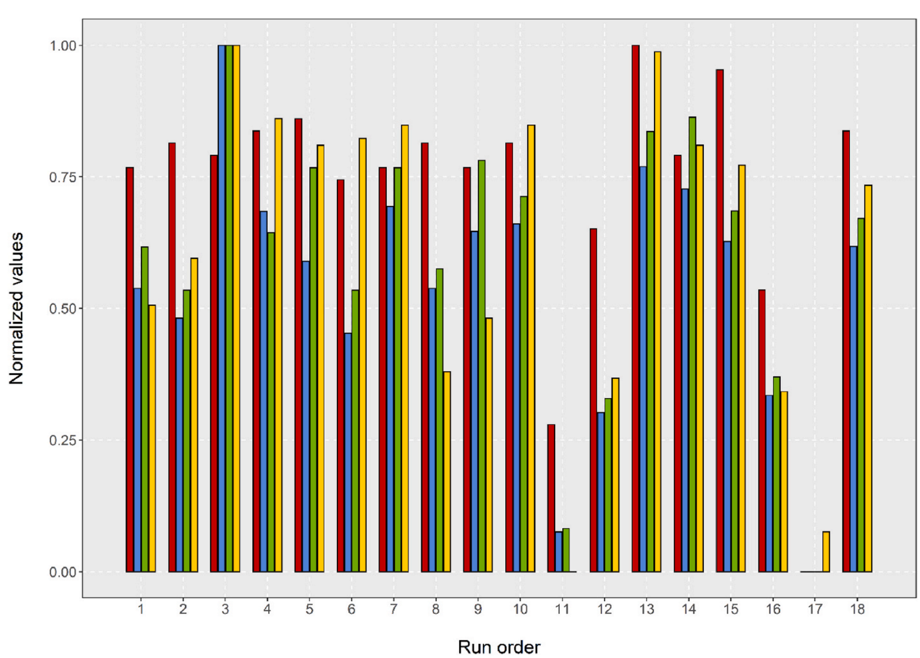

Fig. 1. Bar chart of normalized responses for each experimental run. Each bar is filled with a specific colour as indicated below: extract (red); KI: Kolbach index (blue); FAN: free amino nitrogen (green); AAL: apparent attenuation limit (yellow). (For interpretation of the references to colour in this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

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
	$\Pr(>\lvert t\rvert)$	<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
	$\Pr(>\lvert t\rvert)$	<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
	$\Pr(>\lvert t\rvert)$	<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
	$\Pr(>\lvert t\rvert)$	<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.

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 Mitteleuropaische 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.

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 4
Summary statistics for each model.

	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
R^2	0.9556	0.9916	0.9762	0.7776
Adjusted R^2	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.

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.

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 R^2 and adjusted R^2. The coefficient of determination R^2 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 R^2 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 R^2 were considered.

Regression coefficients with a p-value greater than 0.05 were discarded. Selected models presented adjusted R^2 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

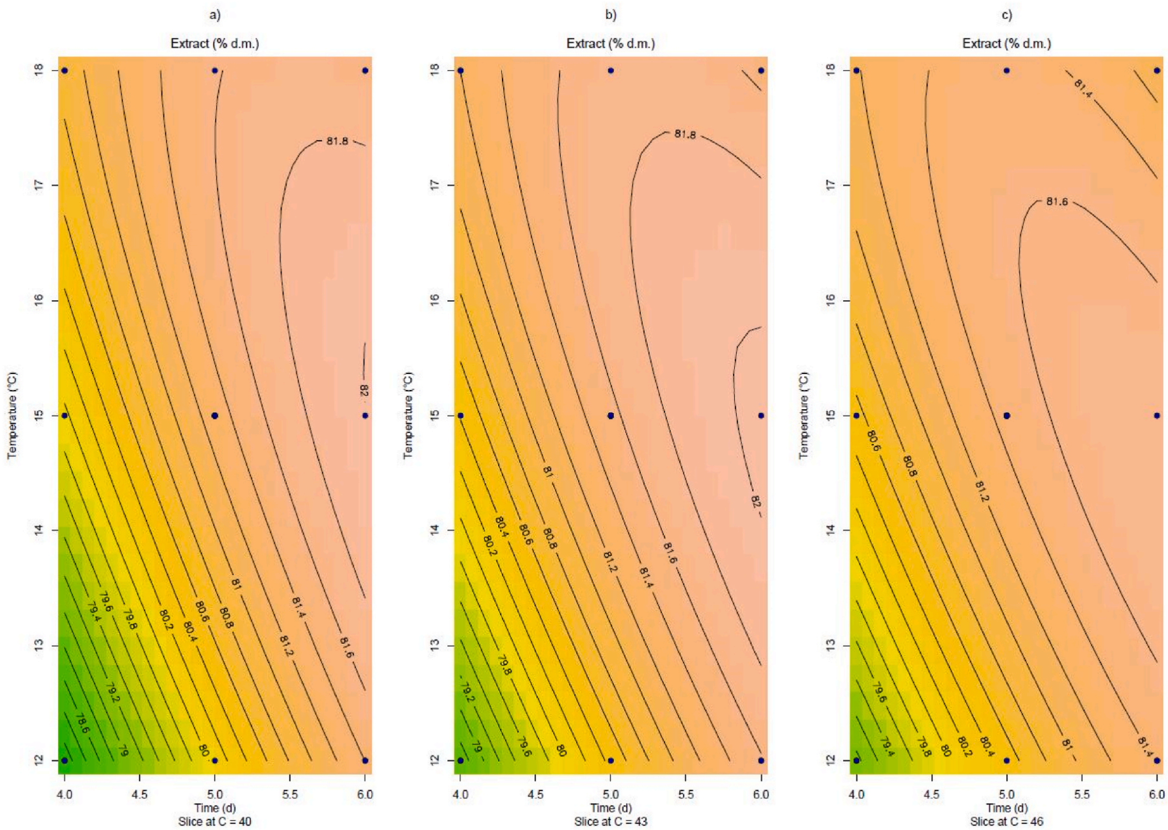

Fig. 2. Contour plots of the predicted extract for different germination times and temperatures. The degree of steeping C is held at a different fixed level in each subplot: a) C at 40 g/100 g; b) C at 43 g/100 g; c) C at 46 g/100 g.

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).

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):

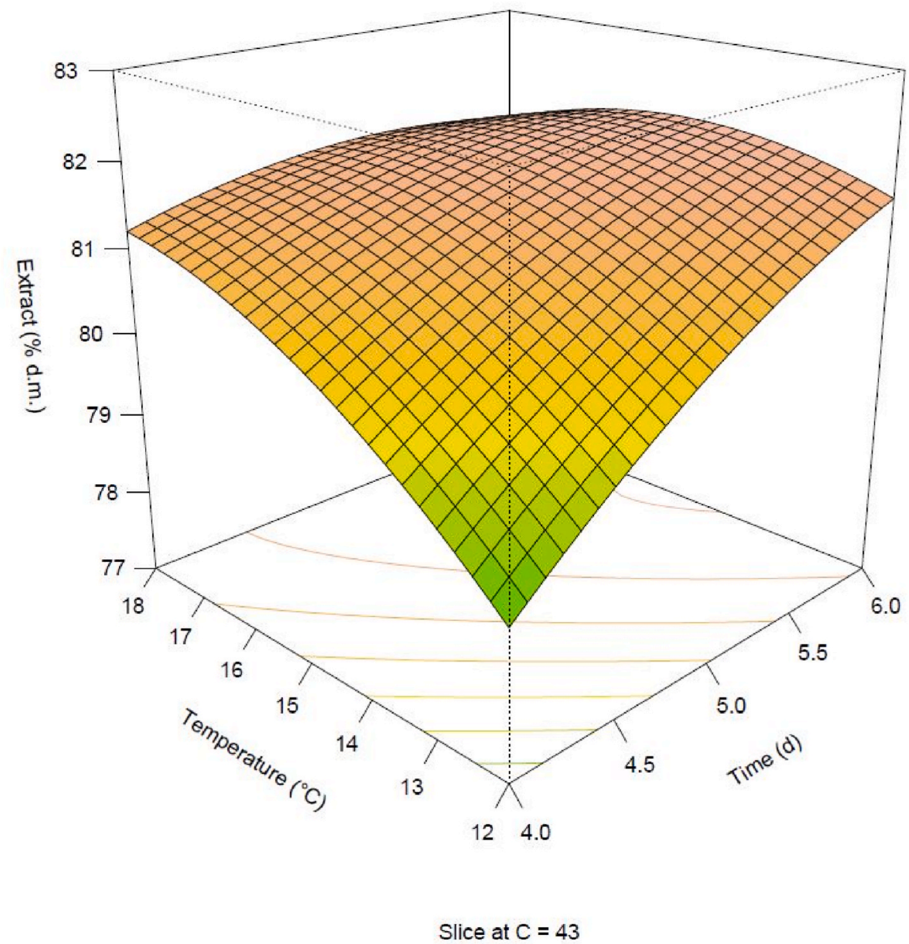

Fig. 3. Influence of germination time and temperature on the predicted extract. The degree of steeping C is held at 43 g/100 g.

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

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.

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 gto 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).

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

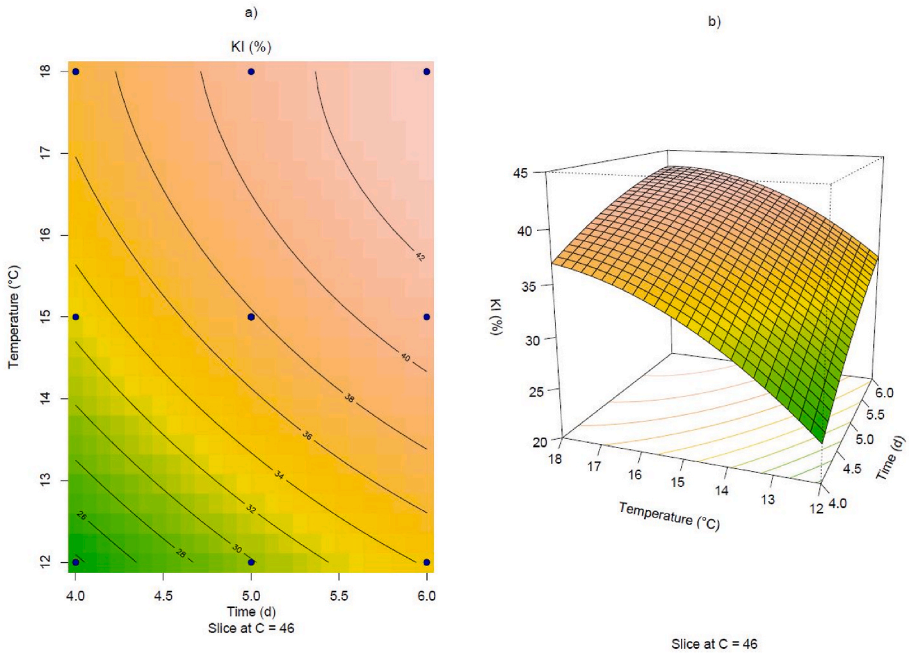

Fig. 4. Contour (a) and surface plot (b) for the influence of germination time and temperature on the Kolbach index (KI). The degree of steeping C is set as 46 g/100 g.

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:

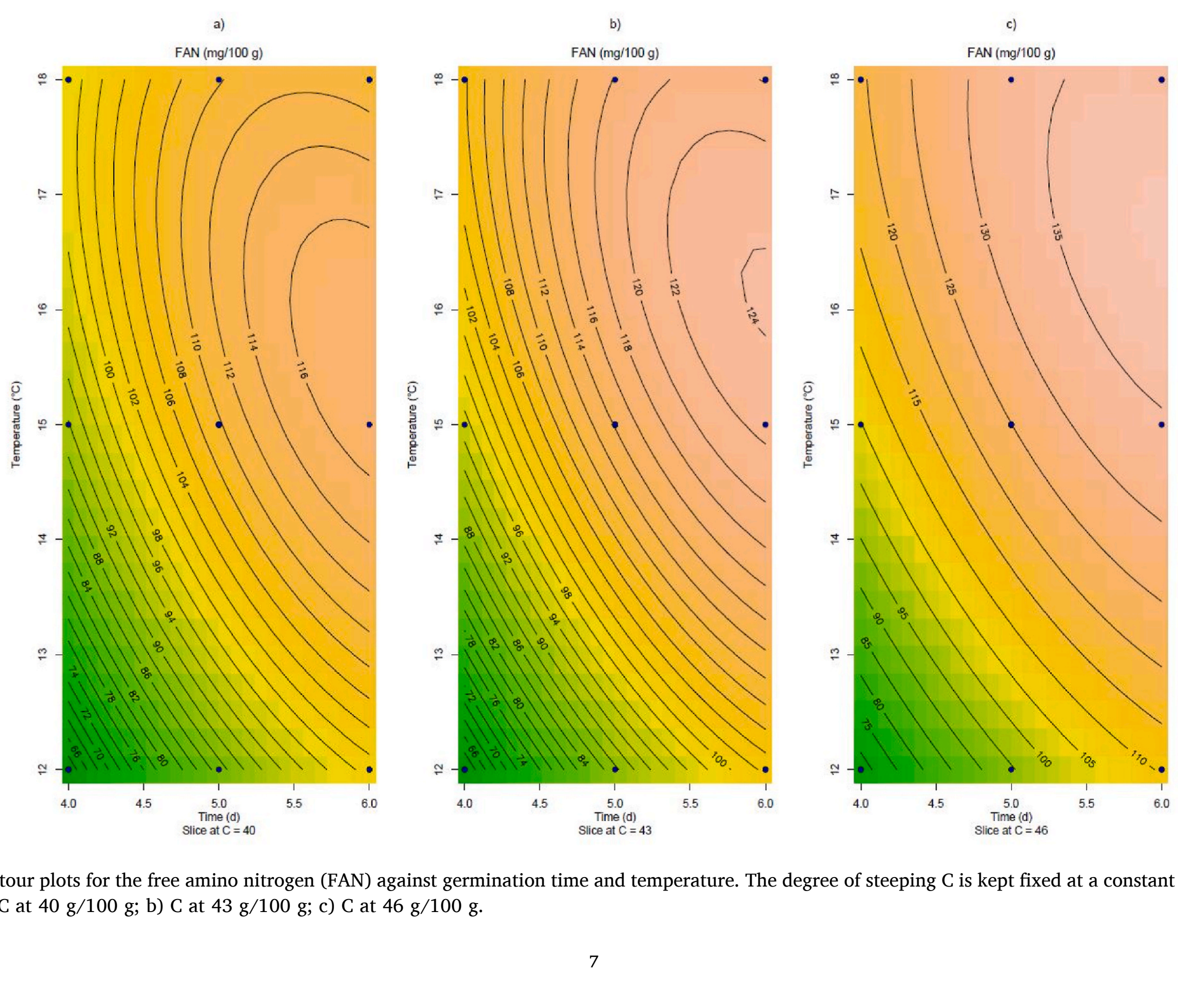

Fig. 5. Contour plots for the free amino nitrogen (FAN) against germination time and temperature. The degree of steeping C is kept fixed at a constant level in each subplot: a) C at 40 g/100 g; b) C at 43 g/100 g; c) C at 46 g/100 g.

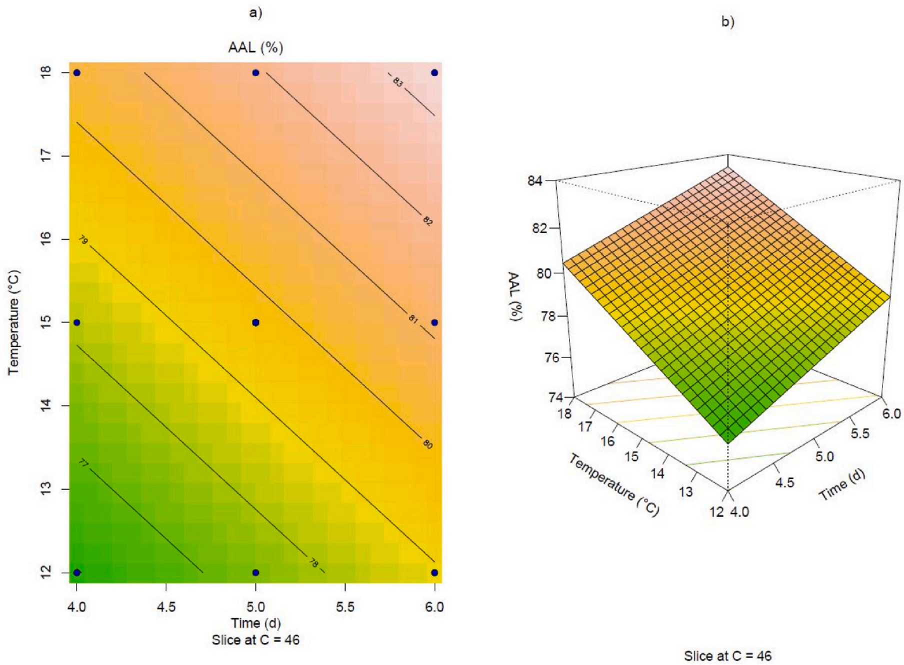

Fig. 6. Contour (a) and surface plot (b) for the apparent attenuation limit (AAL). The degree of steeping C is held at 46 g/100 g.

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

The adjusted R^2 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).

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 (Narziβ, 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 R^2 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.

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 (Narziβ, 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

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.

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.

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 $0 \leq d_i \geq 1$. 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.

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.

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

Table 6
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.

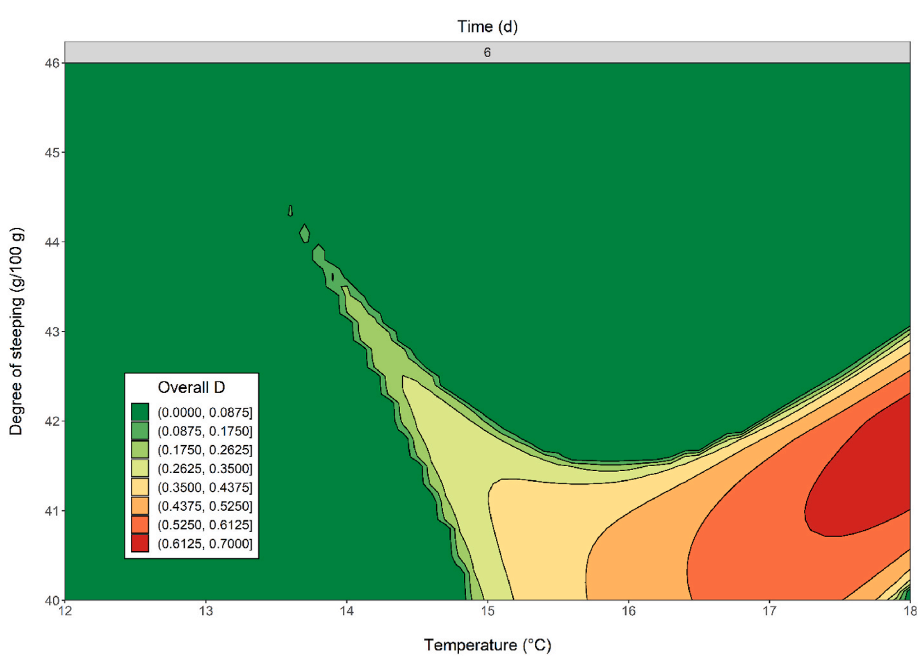

Fig. 7. Contour plot of the overall desirability D. The germination time is held at its optimum level (6 days) and it is represented in the upper part of the graph. Different colours refer to various ranges of overall desirability, as specified in the legend: from dark green to red colour there is an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

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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.114242.

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