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Course code and name:	**B91RS - MSc Research Project - 2022-2023**
Type of assessment:	**Individual**
Coursework Title:	**Effect of Germination Time and Curing Temperature on Tritordeum Malt for Low Wine Aroma**
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Title: Effect of Germination Time and Curing Temperature on Tritordeum Malt for Low Wine Aroma

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Degree Program: MSc Brewing and Distilling with Entrepreneurship

Abstract

Tritordeum, a novel grain from the crossbreeding of durum wheat (*Triticum durum*) and wild barley (*Hordeum chilense*), is gaining substantial attention as an alternative in the alcoholic beverage industry. In grain whisky production, the focus of the malting process is primarily on maximizing alcohol yield. The influence of variations in the malting process on the aroma of whisky has been insufficiently explored. This study aimed to investigate the influence of different germination times (96-144h) and curing temperatures (60-100°C) in micro-malting for tritordeum malt production, assess their suitability for the distillation process, and explore the impact of tritordeum malt produced under various malting regimes on low wine aroma. The effects of malting parameters on the characteristics of tritordeum malt and the low wine volatile compounds were evaluated using the response surface modeling. With an extended germination period, friability, wort volume, and fermentable sugar contents were expected to be increased. The fermentation efficiency using distiller's yeast showed comparable results to malted barley, and a higher alcohol yield in low wine of tritordeum was observed. The concentration of higher alcohols was similar to that of barley malt, but tritordeum malt exhibited higher levels of esters and phenolic compounds in low wines. The levels of higher alcohols and phenols in tritordeum low wine were considerably different depending on the curing temperature. The fact that different volatile compounds and concentrations were shown according to germination time and curing temperature suggests the effects of malting parameters on tritordeum characteristics and their impact on low wine aroma.

1. Introduction

Tritordeum is a novel hybrid species resulting from the crossbreeding between durum wheat (*Triticum durum*) and wild barley (*Hordeum chilense*), with high tolerance to drought and salinity stress, enabling it to sustain substantial biomass production (Vassiliadis, 2022; Yousfi *et al*., 2010). Additionally, Tritordeum has a reduced environmental footprint through increased nitrogen absorption and enhanced water utilisation efficiency (Martin *et al*., 1999). It has similar characteristics to wheat and rye like lacks husk (Figure 1), and relatively high protein and arabinoxylan contents (Shewry *et al*., 2023; Baillière *et al*., 2022; Suchowilska, Radwiec and Wiwart, 2021a). The most significant characteristic is its notably higher diastatic power and β-amylase activity of tritordeum is higher than malted barley, but the α-amylase activity is lower than other grains (Table 1). Raw tritordeum is expected to have a high fructose content according to a study by Demeester *et al*. (2023).

Tritordeum malt for brewing has become commercially available. In two studies, it was observed that beer produced with tritordeum malt exhibited comparable alcohol levels and other quality parameters to beer made with malted barley (Yding *et al*., 2022; Zdaniewicz *et al*., 2020). Especially, it was confirmed that a longer germination period led to higher α-amylase activity (Yding *et al*., 2022). This highlights the potential of tritordeum malt for use in spirit production, indicating its

ability to provide a unique aroma due to its distinct composition compared to other grains.

Table 1. Characteristics of tritordeum, wheat, barley, and barley malt

	Tritordeum	Wheat	Barley	Barley Malt
Husk[a] (%dm)	-	-	11.6	10.6
Starch[a] (%dm)	64.0	68.6	62.7	55.0
Protein[a] (%dm)	15.8	14.8	10.7	10.6
Fat[a] (%dm)	1.7	1.5	2.0	2.2
β-glucan[a] (%dm)	0.45	0.48	3.66	0.27
Diastatic power (°WK)	260[b]	152[a]	251[a, b]	363[a]
α-amylase[a] (CU/g dm)	0.09	0.13	0.03	54
β-amylase[a] (B3U/g dm)	33.5	32.1	18.8	16.2

Mean value
[a]Baillière *et al*. (2022)
[b]Demeester *et al*. (2023)

The malting process is essential to contribute to flavours and aromas of the malt and final products (Yin, 2021; Prado, Gastl and Becker, 2021). The process consists of steeping, germination, and kilning. Germination is critical for enzyme production, breaking down carbohydrates and proteins into fermentable sugars and amino acids, thus leading to the formation of flavour components. During germination, lipid oxidation gives rise to volatile compounds, while phenolic compounds degrade into water-soluble molecules like ferulic acid, contributing to the characteristic aroma (Yin, 2021). Longer germination leads to the elevation of enzyme activities and the synthesis of antioxidants, serving as a protective mechanism against oxidative damage to the grain (Lekiing and Venkatachalam, 2020; Farzaneh *et al*., 2017). The sugars and amino acids in green malts act as ideal substrates for generating aromatic characteristics when exposed to heat during kilning (Prado, Gastl and Becker, 2023; Yin, 2021). Furthermore, the concentration of various aldehydes continues to rise throughout the germination and still remains in the roasting stage (Dong *et al*., 2013). The kilning temperature significantly influences enzyme activities, sugar, and amino acid consumption, and, most importantly, contributes to the development of flavours and aromas (Huang *et al*., 2016). Higher kilning temperatures intensify both the Maillard reaction and caramelization processes (Yin, 2021; Samaras *et al*., 2005), resulting in the production of important volatile compounds such as furans, pyrroles, and pyrazines, which play a vital role in shaping the aroma of malt and whisky (Prado Gastl and Becker, 2023; Marčiulionytė *et al*., 2022; Boothrovd *et al*., 2014).

The spirit market has been experiencing growth globally and sustainability has become a main consideration in the industry facing of climate change (IWSR, 2023). Customers are interested in exploring diverse product categories and experiencing new, high-quality tastes. Additionally, they have prioritised products that show a dedication to sustainability. In response to these trends, whisky producers make efforts towards achieving sustainability throughout their entire production process, from raw material selection to sourcing. Furthermore, they focus on product differentiation by using new raw materials to offer customers novel experiences.

However, there is a lack of research on the contribution of grains to the aroma in whisky production unlike brewing (Wanikawa, 2020). Studies have focused on developing enzymes to improve malt quality by breaking down starch and β-glucan due to their high ethanol yield and ease of handling during the mashing process (Bathgate, 2016; Green *et al*., 2015; Agu *et al*., 2009). Additionally, in the brewing industry, studies have been conducted on using alternative grains to introduce new

flavours, but not much research has been done on this topic in whisky production. While research has been conducted on yeast and pot still, which influence whisky flavour during fermentation or distillation (Waymark and Hill, 2021; Harrison *et al*., 2011), there is limited study on the impact of congeners generated due to differences in the malting process on whisky aroma.

This study examines the impact of altering germination time resulting in varying enzyme activities and subsequent production, on the compounds developed during the kilning process, particularly the curing stage, in tritordeum for distillation. The aim is to evaluate which malting regime is suited for distillation and to assess the characteristics of low wine made from tritordeum malt to understand how compounds produced during malting process influence the aroma of the low wine, facilitating the determination of an optimal cut point for new make spirits production. To achieve this, the study analyses the characteristics and quality of tritordeum malt produced by varying germination times and curing temperatures. Moreover, it examines how tritordeum malts interact in the low wine production process and whether the final product possesses distinctive aromas.

Figure 1. Images of barley malt (A), wheat malt (B), rye malt (C), and tritordeum malt (D).

2. Materials and Methods

2.1. Raw materials

The raw tritordeum of the variety Coique (HT444) was supplied by Vivagrain S.L. (Barcelona, Spain). As a reference, extra pale malted barley was obtained from Crisp Malt (Norfolk, UK).

2.2. Analysis of Raw Tritordeum

Prior to malting, germinative energy and water sensitivity of tritordeum were assessed following the EBC 3.6.2 method in triplicate. Moisture content was measured according to the EBC 3.2 and thousand corn weight was measured with the weight of 100 grains multiplied by 10. The percentage of grains screened with a size larger than 2.2mm was determined by passing 400g of grains through a sieve, and then measuring the weight of the remaining grains.

2.3. Micro-malting

Grains (400g) were divided into individual baskets and malted at the Steep-Germinator Curio Malting MMSG (Curio, Milton Keynes, UK) and kilned in a Curio Malting MMK-kiln. The steeping regime and germination temperature for tritordeum were advised by Boortmalt (Antwerp, Belgium). The steeping regime was consistent for all samples, followed by 8h of wet, 15h of air-rest, and 12h of wet at 15°C. The kilning regime was adapted from the study (Yding *et al*., 2022) and modified to give a change in curing temperature. Germination time and curing temperature parameters were determined using Design Expert 22.0.3 (Stat-Ease Inc., Minneapolis, MN, U.S.A) to optimise the sampling points. The variables, including germination times of 96h, 108h, 120h, 132h, and 144h, and curing temperatures of 60°C, 70°C, 80°C, 90°C, and 100°C, were evaluated based on 19 data points. The experimental design included replicates at the vertices of the model and triplicates at the center point.

For each data point, grains were germinated at 17°C for the specified germination time (96h, 108h, 120h, 132h, and 144h). Subsequently, the kilning regime was followed at 55°C for 16h, followed at 60°C for 4h, and then the final curing temperature of 60°C, 70°C, 80°C, 90°C, or 100°C for 4h each. The kilning temperature was ramped up at a rate of 1°C/min. After the completion of malting, sprouts and rootlets were removed from the malted grains. To address issues with small kernel sizes and dried rootlets and dust adhering to the malt, a 1.8mm sieve was used to retain the small grains and remove the adhered rootlets from the malt. All the produced samples were kept at room temperature before analysis.

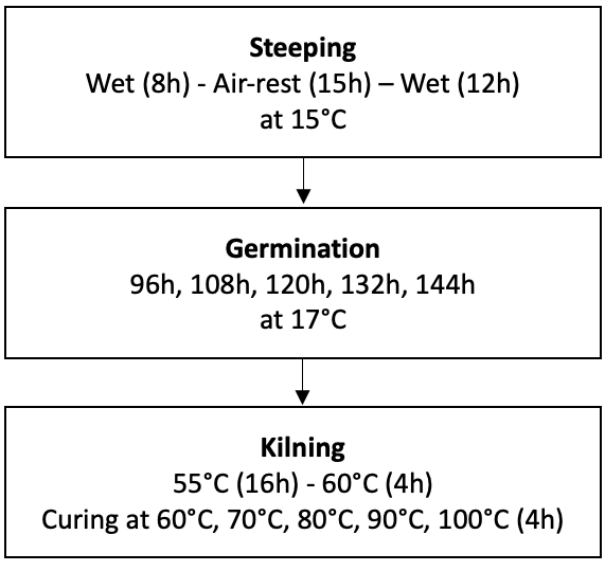


Figure 2. Tritordeum malting regime.

2.4. Wort production and fermentation

Tritordeum malt was milled using a DLFU Bühler Miag disc mill (Uzwil, Switzerland) set to a gap of 0.7mm. Milled grains (100g) were mixed with 310ml of 65°C distilled water and mashed at 65°C while constantly stirring for 60min using a CM4 Mash bath (Canongate Technology Ltd., Edinburgh, U.K). After 60min, the samples were cooled to 20°C and adjusted to 450g with distilled water (20°C). The samples were then filtered through 113V fluted filter paper (Whatman, Kent, UK), and the first 50ml of filtrate was returned to the filter. Funnels containing the mash samples were covered with aluminum foil to prevent evaporation and filtered overnight at 7-8°C to obtain enough wort. Since the volume of wort produced from tritordeum was insufficient for distillation, mashes were duplicated to allow for consolidation of wort prior to fermentation. The produced wort was frozen prior to fermentation. A barley malt reference was also processed as described above.

Before the yeast pitching, the frozen wort (samples and reference) was defrosted at 30°C for 2h and stirred to properly mix the wort. The gravity of all wort samples was adjusted to 1.0550. The wort (190ml) was pitched with 1g/L of N379 dry yeast (Lallemand, Canada) into a 250ml glass bottle, agitated manually, and fermented fitted with an airlock for 72h at 30°C. The specific gravity of wort and wash samples was measured with an Anton-Paar DMA 35 density meter (Graz, Austria).

2.5. Low wines production

Fermented wort was frozen and defrosted in a water bath at 30°C before distillation. The wash (180ml) was distilled with 3 drops of 0.02mL/L FD20P silicone-based anti-foam (Murphy & Son Ltd, Nottingham, UK) using a round bottom flask (250ml) and an Electrothermal CMU1000/CE heating mantle (Electrothermal, Essex, UK). The heating mantle was set to 6 until the wash boiled and adjusted to 5. The rolled copper mesh (9cm x 7cm and 7cm x 7cm) was inserted into the head and front of the condenser, respectively (Figure 3), to remove sulfur compounds through contact between copper and vapour. The low wine was distilled until the distillate reached 1% ABV (measured with Anton-Paar DMA 35 density meter).

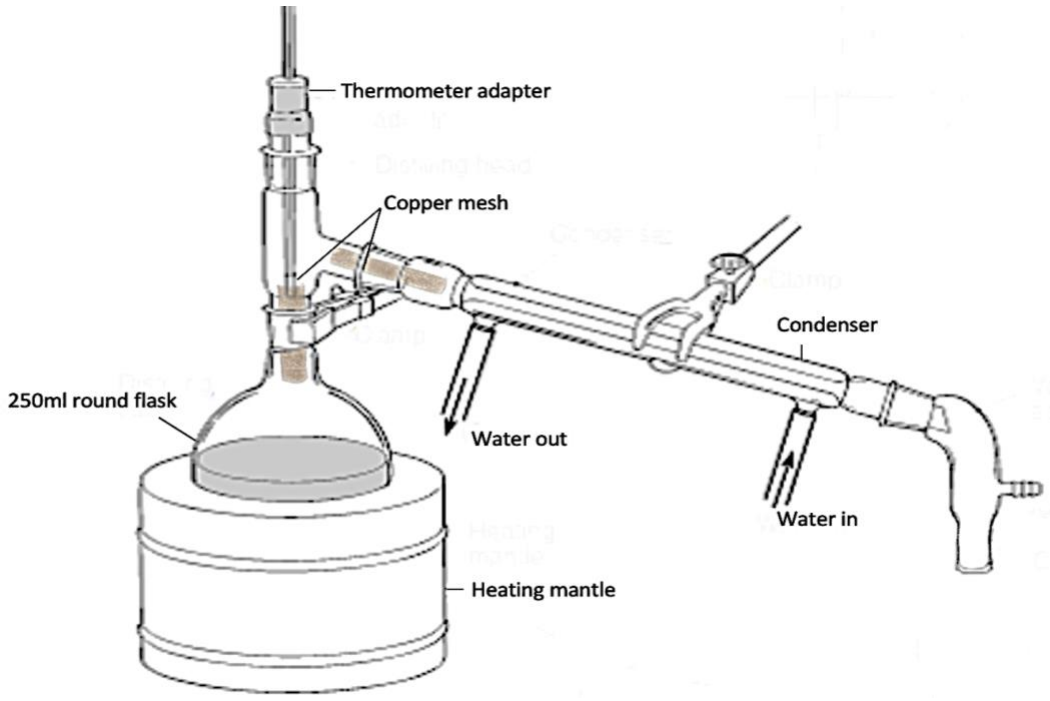


Figure 3. Diagram of wash distillation. Modified from Tro and Norton (2017).

2.6. Analysis of malt and wort

The malting weight loss of tritordeum was calculated using the following formula:

$$\textbf{\textit{Malting weight loss}} = \frac{\textbf{\textit{Weight of grain used for malt} - \textbf{\textit{weight of polished malt}}}}{\textbf{\textit{Weight of grain used for malt}}} \times \mathbf{100}$$

Analysis of tritordeum malt and extra pale malt was performed following the European Brewery Convention (2018) methods. The analysis included measurements of moisture content of malt (4.2), friability (4.15), wort pH (8.17), and wort free amino nitrogen (FAN; 8.10.1). Samples for FAN analysis were analysed in duplicate. The malt colour was measured by adjusting EBC 4.7.1, after recirculating the first filtrate during wort production and by collecting samples from the first 50 ml. The sugar profile of wort was determined by centrifuging wort (1.5ml) at 13.3kg for 5 min, then collecting 1ml of supernatant for HPLC analysis.

2.7. Analysis of low wine congeners

The HS-GC-FID method was used to determine higher alcohol and ester compounds. The analysis utilised an Agilent 7820 A gas chromatograph with a 7697A headspace injector and simultaneous ECD/FID detection (Agilent, Santa Clara, CA, USA). A 100µL internal standard solution (4000mg/L of heptanone in ethanol) was added to low wine samples (10ml) diluted with distilled water to 9.5% ABV to a vial (20ml). The HS injector maintained equilibrium at 85°C for 15min, followed by 0.5min injection (loop temperature at 85°C, transmission line at 100°C, and injector at 210°C). An Agilent DB-wax column (30m x 0.25mm x 0.25µm) was employed. The column oven temperature was initiated at 40°C for 0min, then ramped to 60°C at 5°C/min, further ramped to 250°C at 8°C/min, and held for 8min, resulting in the total run time of 35.75 min. The FID detector operated at 270°C. Data processing was carried out using the Agilent Chemstation data handling system.

Phenolic compounds were analysed using a Shimadzu LC-20 modular HPLC system, which consisted of a degasser, LPG quaternary pump, autosampler, and detectors (PDA, RID, and Fluorescence) with a Shimadzu LC-10 column oven (Shimadazu, Kyoto, Japan). A low wine sample (1ml) was used, and the eluents were 100% H2O (A) and 100% MeCN (B). The gradient program initiated at 10% of B for 0min, then increased to 40% of B in 7min, followed by a ramp to 80% of B in 2min, and a subsequent ramp to 95% of B for 6min, with re-equilibration for 5min (flow rate: 1 ml/min; the total run time: 20min). The Phenomenex Prodigy 5µm ODS(2) 150 Å (Phenomenex, Torrance, CA, USA) column was used, and the oven temperature was maintained at 40°C. Fluorescence detection utilised an excitation UV 272nm and an emission UV 298nm.

2.8. Statistical analysis

The 19 points D-optimal response surface design was created using Design Expert 22.0.3 for statistical analysis, considering the two factors of germination time and curing temperature. Due to low wort recovery in some samples, some data points were missing when producing models for wort and low wines quality characteristics. The measured values were fitted to a suitable polynomial curve in the model, and the statistical significance of the model was determined using two-way analysis of variance (ANOVA). A statistically significant level was set at 5% (p-value<0.05). When the model fit was not significant, an appropriate regression model was selected considering where the p-value of the lack of fit was not significant (p-value>0.05) or R^2 was close to 1.

3. Results and Discussion

3.1. Raw tritordeum quality

The evaluation of the suitability of raw tritordeum for the malting process was conducted. The moisture content of the raw tritordeum was measured at 11.3% (Table 2), which is generally considered an appropriate value (<13.5%) that is unlikely to be contaminated by external microorganisms during storage (Yin, 2021). Initial moisture content influences the rate of water uptake during steeping, and drier grains require longer steeping times (Brookes, Lovett, and MacWillinam, 1976). Moreover, the water uptake is influenced by kernel size (Brookes, Lovett, and MacWillinam, 1976), with tritordeum exhibiting a narrower width of 2.3mm compared to barley, indicating a smaller kernel size (Table 2; Figure 1). Assessing the viability of raw grains is crucial to ensure successful malt modification. The germinative energy of tritordeum was observed at 97.3%, indicating a high level of viability and the potential for effective malt production. Furthermore, the water sensitivity value of 0.3% suggested that tritordeum is not highly sensitive to moisture, highlighting its capability to germinate well even during longer wet periods.

Table 2. Characteristic and quality of raw tritordeum

	Raw tritordeum
Moisture content (%)	11.3 ± 0.3
Length (mm)	8.7 ± 0.6
Width (mm)	2.3 ± 0.3
Thousands grain weight (g)	27.4 ± 1.2
> 2.2mm screened grain (%)	43.7 ± 3.7
Germinative energy, 4ml (%)	97.3 ± 1.2
Water sensitivity (%)	0.3 ± 1.2

Mean ± Standard deviation (n=3)

Table 3. Summary of characteristic and quality of extra pale and tritordeum malts for low wine production

	Extra pale malt[a]	**Minimum value**	**Maximum value**	**Model fit[b]**	**Model F-value**	**Model p-value**	**Model R^2**	**Lack of Fit**
		Tritordeum malt						
Malting weight loss (%)	-	15.3	22.7	Quadratic	9.84	0.0005	0.7910	0.2420[c]
Moisture content (% m/m)	7.04±0.3	3.40	7.78	Quadratic	7.71	0.0014	0.7478	0.6073[c]
Friability (%)	95.2±0.6	79.5	94.4	2FI	5.13	0.0122	0.5065	0.8881[c]
Colour of malt (EBC)	6.6±0.1	14	38	Quadratic	5.96	0.0044	0.6964	0.1065[c]
		High gravity wort						
Wort volume (ml)	454±3	172	411	Quadratic	1.34[c]	0.3298[c]	0.4271	0.0584[c]
Wort gravity	1.0714± 0.0001	1.0580	1.0649	Quadratic	2.50[c]	0.1097[c]	0.5816	0.2023[c]
Wort pH	6.0±0.1	6.0	6.3	Quadratic	3.30[c]	0.0574[c]	0.6469	0.2859[c]
Wort FAN (mg/L)	300.4±23	122.6	240.6	Cubic	7.13	0.0217	0.9277	0.2823[c]
Fermentable sugar (g/L)	153.1±9.1	97.9	129.8	Cubic	5.47	0.0585 [c]	0.9248	0.0376

				Wash and low wine				
Final gravity	1.0036± 0.0001	1.0032	1.0074	Cubic	4.62	0.0534[c]	0.8926	0.2174[c]
Wash pH	3.6±0.0	3.5	3.7	Quadratic	2.01[c]	0.1714[c]	0.5273	0.9689[c]
Low wine (% ABV)	16.3±0.3	14.6	18.0	Quadratic	2.01	0.1817[c]	0.5565	0.3654[c]

[a]Mean ± Standard deviation (n=3)
[b]Model fit was optimised and in the case where the *p*-value was not significant, the lack of fit value considered for its configuration. The statistical values were determined using ANOVA (Design Expert 22.0.3).
[c]not significant (*p*-value>0.05)

3.2. Tritordeum malt characteristics

Malting loss has a direct impact on the economics of malting production. It increased with longer germination period (G) and higher curing temperature (K) (Figure 5A). The result showed that germination time was significant for malting loss ($p<0.0001$). Longer germination times result in more biomass being produced in the kernels due to proteolysis and degradation of the endosperm by enzymatic activation which leads to more malting loss (Briggs, 1998). The lowest value was 15.3% at G96h and K60°C, and the highest value was 22.7% at G144h and K100°C (Table 3; Figure 5A). This is higher than the average malting loss of pale malts of 13-14% (Briggs, 1998). Depending on the germination period, rootlets grow less or more, but particularly grains without husk like tritordeum showed a higher loss than covered grains because of a proportion of acrospires (Edney and Langrell, 2004; Briggs, 1998) (Figure 4). Loss of water at high curing temperatures also resulted in the value increased.

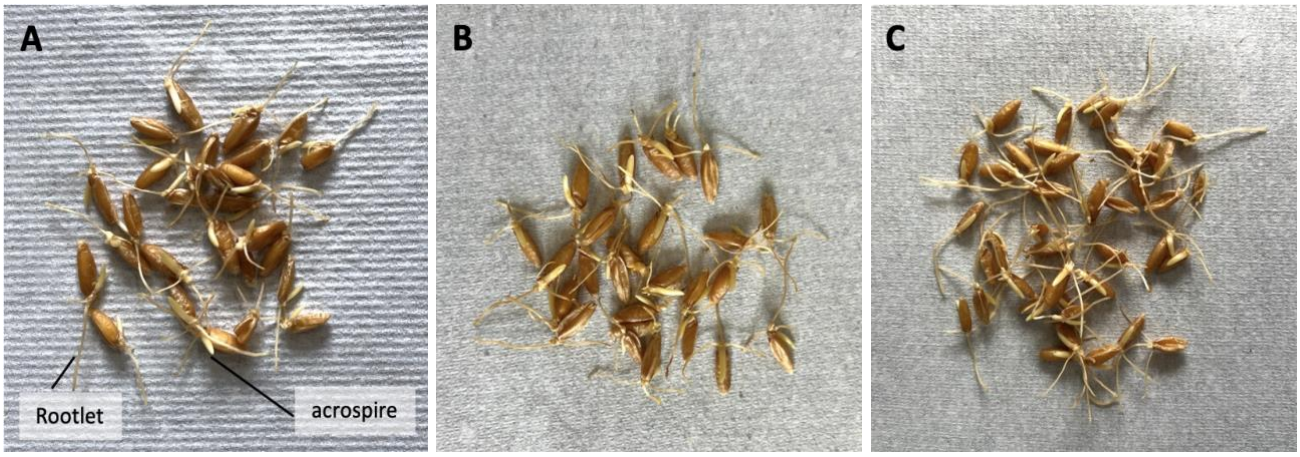


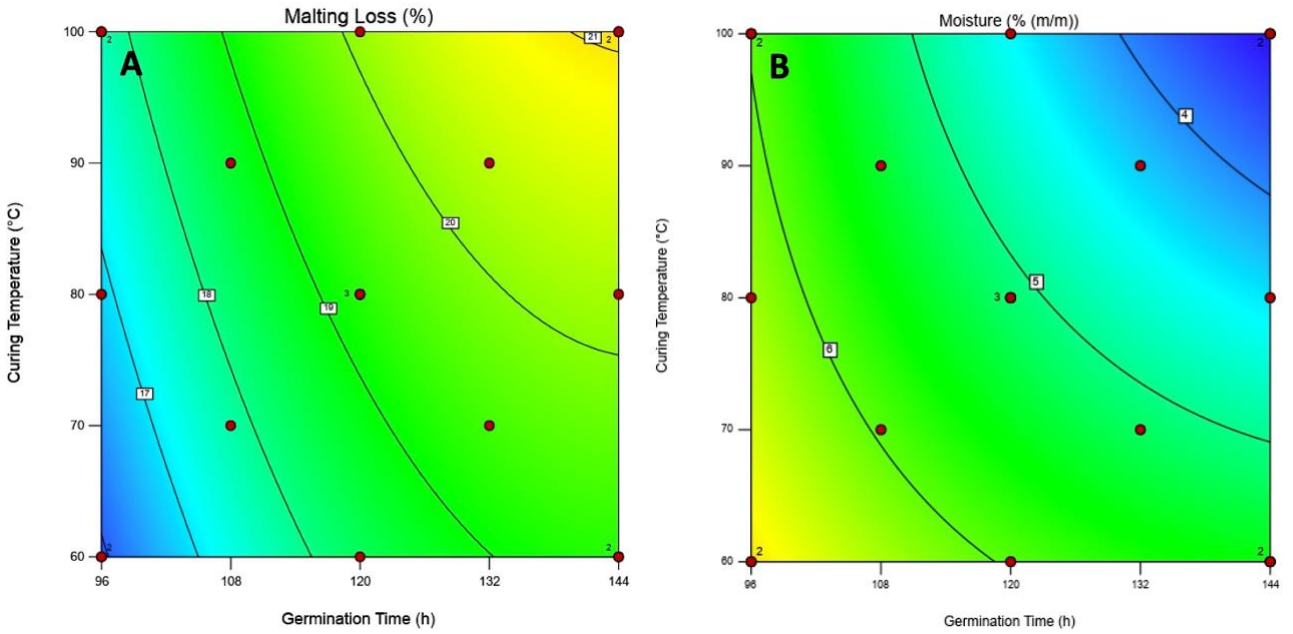
Figure 4. Green malts of tritordeum. (A) G96h; (B) G120h; and (C) G144h.

The moisture content decreased with an extended germination period and higher curing temperature (Figure 5B). The moisture contents of tritordeum malt ranged from 3.40 to 7.78% m/m (G144h and K100°C; G96h and K60°C, respectively; Table 3), which were comparatively lower than barley malt (Table 3). The values observed at K80°C ranged from 4.93 to 5.80% m/m, which was higher than that in the previous Yiding *et al*. (2022) study (approximately 4% m/m). This increase could be attributed to the influence of longer steeping time and higher germination temperature. However, three data points (1 point at G96h and K60°C; 2 points at G 96h and K100°C), which underwent germination in the same bay and remained in the kiln bay over the weekend, showed different patterns. Despite the general tendency of moisture content to decrease and friability to increase with rising curing temperatures within the same germination period, these three points did not conform to this trend observed in G96h. Notably, the moisture content of K100°C was recorded

at 5.80–6.84% m/m, higher than that of K80°C (5.41% m/m) in germination 96h. This discrepancy was also evident in friability, malt colour, and FAN content among other samples. However, surprisingly, no significant difference was observed in fermentable sugar contents.

Friability indicates how well the endosperm of grain is modified and is typically considered well-modified when it is >80% (Yin, 2021). Friability is influenced by the texture of the kernel, enzyme activities, and malting conditions (Yin, 2021). With an extended germination period, the friability of tritordeum malt demonstrated an increase, which could be confirmed to increase with the higher curing temperature as malt dried (Figure 5C). The lowest value was observed in G96h and K60°C (79.5%), and values for G144h were all >90% (Table 2; Figure 5C). The maximum value was 94.4% (G144h and K100°C), slightly lower than the 95.2% of the control, extra pale malt. These results indicate that compared to barley, tritordeum requires a longer germination period to be adequately modified. This suggests a connection between the increase in α-amylase and the degradation of starch in the endosperm, as shown in the study (Yiding *et al*., 2022). In terms of homogeneity, both malted tritordeum and extra pale malt showed a 100% value.

The malt colour of tritordeum (minimum value: EBC 14; maximum value: EBC 38) was more than twice as high as that of extra pale malt (EBC 6.6) (Table 3), and notably increased with curing temperatures above 80°C (Figure 5D). The reason for showing values higher than the original EBC is due to the collected samples during the high gravity wort production. Tritordeum contains a higher amount of carotenoids, which are colour pigments found in plants and impart yellow or orange (Martín *et al*., 1999). The carotenoid content known for its poor water solubility, in tritordeum, showed higher pigment retention at elevated temperatures (Mellado-Ortega and Hornero-Méndez, 2017). This indicates the influence of grain particle type and curing temperature on malt colour (Girón-Orozco *et al*., 2023). Throughout the kilning process, temperature affects colour and flavour through a non-enzymatic browning reaction known as the Maillard reaction, resulting in a darker colour, especially at higher temperatures (Prado, Gastl and Becker, 2021).



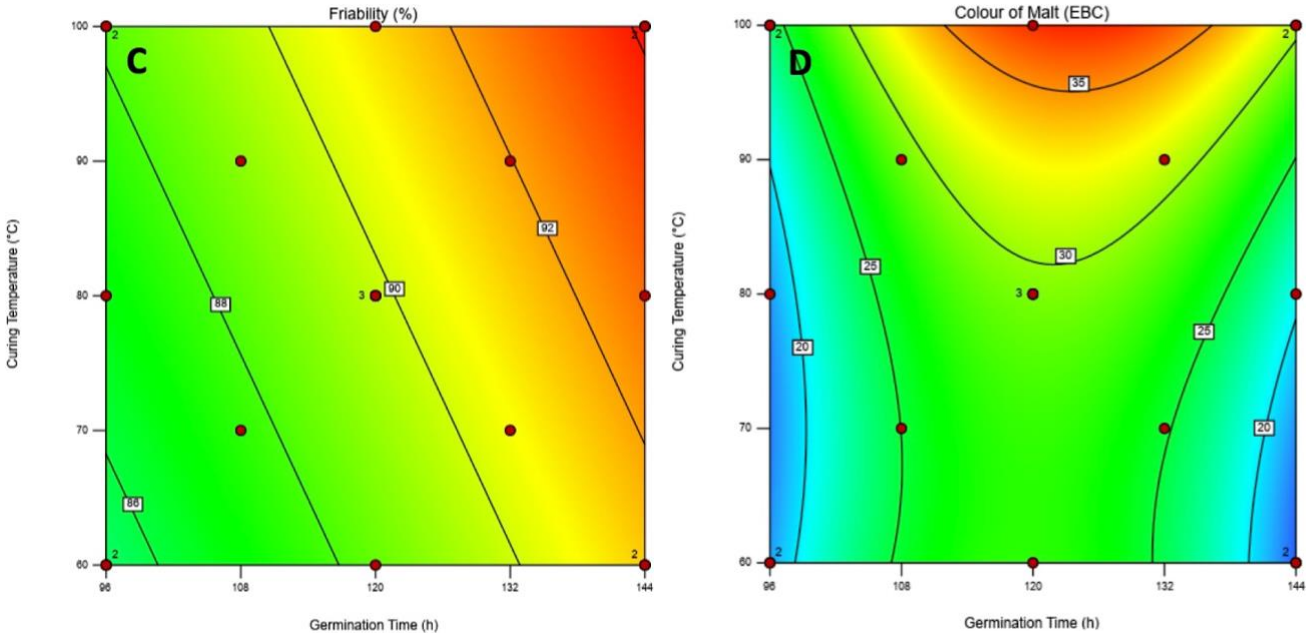


Figure 5. Tritordeum malt quality. (A) Malting loss (%); (B) Moisture content (% (m/m)); (C) Friability (%); (D) Colour of malt (EBC). As the colour goes from blue to red, it indicates the value increases. The red dots indicate data points.

3.3. High gravity wort quality for distillation

All the values were measured by combining the wort produced in duplicate (samples and extra pale malt). Wort volume recovery showed non-significant result based on two variables (germination time and curing temperature) (p=0.7423). The lowest value (172ml) was observed at G120h and K80°C, while the highest value was seen at G96h and K60°C (411ml) (Table 3). In contrast, the wort yield of extra pale malt remained consistent and was higher than that of tritordeum malt (454±3ml) (Table 2). This could be attributed to the absence of husk in tritordeum, along with its high levels of arabinoxylan and protein contents, potentially causing filtration issues (Shewry *et al*., 2023). The degradation of arabinoxylan follows a different pattern from the decrease of β-glucan that occurs as germination progresses. In malted barley, there was a noticeable reduction in the high-molecular-weight fraction of arabinoxylan only within the first 2-3 days of germination (Yin, 2021; Eom and Lee, 2008). During germination, xylanase activity in barley increased until the third day, then decreased before rising again (Kanauchi *et al*., 2013). In the case of rye malt, a rapid increase in wort arabinoxylan content was observed on the fourth day of germination (Wang *et al*., 2018). The optimal temperature of endoxylanase is 55-60°C, however, knowledge about xylanase during malting remains limited to date (Geißinger, Gastl and Becker, 2022; Yin, 2021). Based on this, there might have issues with arabinoxylan degradation and filtration at G120h and K80°C. Excluding this, there were no filtration issues observed as in previous studies (Demeester *et al*., 2023; Yding *et al*., 2022; Zdaniewicz *et al*., 2020).

The gravity of the wort was lower than malted barley (Table 3). This may be due to the small kernel size of tritordeum. The wort gravity showed a graph with a negative correlation to the volume (Figure 7A and B). The highest values were expected to be at G144h but were observed at G120h and K80°C. Incomplete drainage of water in the filter cake at G120h and K80°C may have an impact.

The wort pH is critical in regulating enzyme activity. For the degradation of starch into fermentable sugars using amylolysis enzymes, the optimal pH range is 5.0 to 6.5 (Yin, 2021). Statistically, there

was no significant difference observed among variables (p=0.4786). While Giron-Orozco *et al*. (2021) confirmed that curing temperature did not significantly affect triticale wort pH. However, the graph showed that as the germination period lengthened and the curing temperature increased, the pH tended to decrease (Figure 6C). This indicates that the longer the germination time, the more oxidation of the lipid occurs, and the acidity increases during producing melanoidin on Maillard reaction at higher curing temperature (Yin, 2021). Wort pH showed to be strongly correlated with wort colour (Marčiulionytė *et al*., 2022), and wort colour is also correlated with malt colour. As the malt color increased, the wort pH decreased (Figure 5D; Figure 6C).

FAN content is an important factor in the growth of yeast in the early stages of fermentation. The FAN contents of high gravity wort of tritordeum malt showed a lower value (122.6-240.6mg/L) than that of barley malt (300.4±23mg/L) (Table 3). The result was different from studies in which tritordeum showed higher FAN content due to higher protein than barley (Demeester *et al*., 2021; Zdaniewicz *et al*., 2020). In a previous study, the FAN contents of tritordeum wort applied with a long-wet period in the steeping process showed a lower value than that of malted barley (Yiding *et al*., 2022), so this result may be due to the long wet period of the steeping regime. It showed a decrease when cured at >80°C in G108h-120h (Figure 6D). This decrease could be attributed to the impact of Maillard reactions with sugar contents at elevated temperatures. Cured at >90 °C FAN and fermentable sugars contents were expected to decrease due to Maillard reaction, but in the case of fermentable sugar contents shown in the response surface model, high values were predicted at G144h and K100°C (Figure 6E). This is expected to be a result of not statistically significant model fit (Table 3) due to not including 5 data points and confirmation is required. However, interestingly, the model graph showed an increase in sugar contents as the germination period lengthens in parts of the model where data point coverage was higher (G96h-G132h), also it was predicted that more sugars will be extracted at around K80°C (Figure 6E). Given the higher fermentable sugar contents observed at K80°C in the actual data, it may be extracted efficiency could expect enhanced at K80°C. In general, barley germination takes around 4 days at 16°C (Yin, 2021), whereas tritordeum suggests that longer germination at a higher temperature (17°C) is beneficial for obtaining fermentable sugars.

The minimum value of the final gravity was 1.0032 (G132h and K90°C), which was similar to that for barley malt (1.0036±0.0001) (Table 3). The model graph showed that in shorter germination with higher curing temperatures and longer germination with lower curing temperatures would result in decreased fermentability (Figure 6F). When compared to the control, using distiller's yeast, G144h and K80°C were expected to demonstrate similar fermentability. The pH of tritordeum wash (minimum: pH 3.5; maximum: pH 3.7) also exhibited values similar to malted barley (pH 3.6) (Table 3). Germination time and curing temperature showed no statistically significant impact on wash pH (p=0.4559). The maximum alcohol by volume (ABV) of tritordeum low wine was 18% ABV, surpassing the 16.3% ABV of barley (Table 3). The lowest value was observed at G96h and K60°C (14.6% ABV), while predictions from the model graph indicated the highest values for G144h with curing temperatures ranging from 60°C to 80°C (Figure 6H). Generally, low wine exhibits an ABV of less than 20%, highlighting the potential for tritordeum to enable the production of new make spirit.

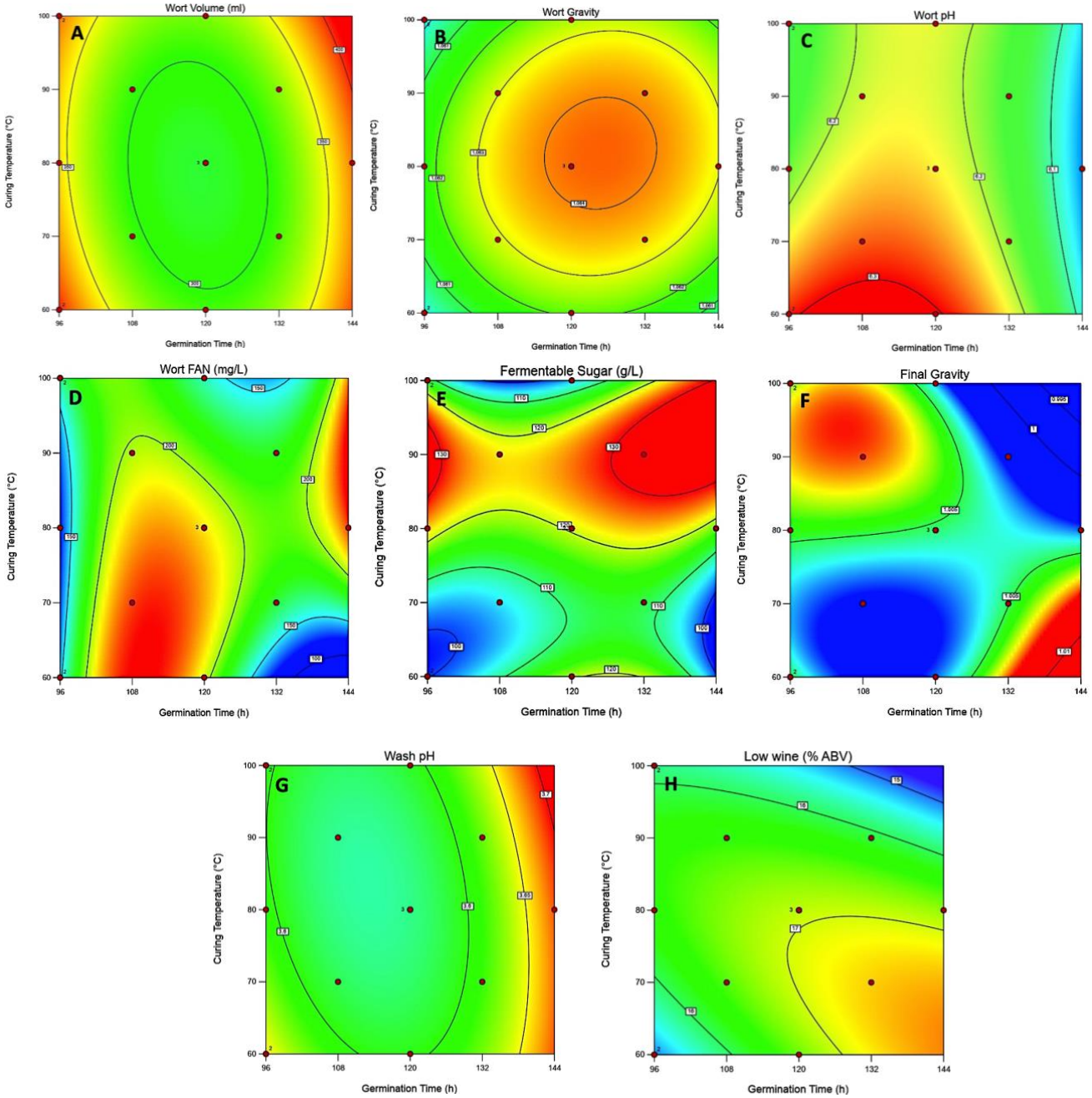


Figure 6. Tritordeum high gravity wort quality and % ABV of low wine. (A) Wort volume (ml); (B) Wort gravity; (C) Wort pH; (D) Wort FAN (mg/L); (E) Fermentable sugar (g/L); (F) Final gravity; (G) Wash pH; (H) Low wine (% ABV). As the colour goes from blue to red, it indicates the value increases. The red dots indicate data points (4 points missing, 5 points missing for fermentable sugar and low wine).

3.4. Aroma compounds in low wine

Table 4. Summary of low wine congeners

	Extra Pale Malt[a]	Tritordeum malt						
		Minimum value	Maximum value	Model fit[b]	Model F-value	Model p-value	Model R^2	Lack of Fit
Higher alcohols								
Isoamyl alcohol (mg/L)	89.2±7.2	63.2	93.1	Quadratic	5.79	0.0149	0.7834	0.1509[c]
Isobutanol (mg/L)	38.6±1.4	25.8	44.0	Quadratic	8.22	0.0052	0.8370	0.1738[c]
1-propanol (mg/L)	9.5±0.6	6.6	14.4	Quadratic	3.77	0.0473	0.7020	0.1525[c]
1-butanol (mg/L)	0.2±0.6	n.d[d]	0.7	-	-	-	-	-
Esters								
Ethyl acetate (mg/L)	6.5±0.6	6.7	11.4	Quadratic	0.99	0.4816[c]	0.3812	0.8169[c]
Isoamyl acetate (mg/L)	0.2±0.0	0.1	0.2	Quadratic	1.75	0.2294[c]	0.5224	0.2228[c]
Ethyl hexanoate (mg/L)	n.d[d]	n.d[d]	0.1	-	-	-	-	-
Ethyl lactate (mg/L)	n.d[d]	n.d[d]	14.2	-	-	-	-	-
Phenolic compounds								
Phenol (µg/L)	182±2	110	153	2FI	2.31	0.1327[c]	0.3865	0.1375[c]
4-vinyl guaiacol (µg/L)	120±104	n.d[d]	287	-	-	-	-	-
4-ethyl guaiacol (µg/L)	27.8±3.9	31.1	54.1	Quadratic	11.24	0.0012	0.8620	0.4264[c]
4-ethyl phenol (µg/L)	1.6±2.8	n.d[d]	6.7	-	-	-	-	-

[a]Mean ± Standard deviation (n=3)
[b]Model fit was optimised and in the case where the *p*-value was not significant, the lack of fit value considered for its configuration. The statistical values were determined using ANOVA (Design Expert 22.0.3).
[c]not significant (*p*-value > 0.05)
[d]not detected

Higher alcohols and esters are key fragrance elements that yeast activity generates during fermentation, resulting in the development of fruity and floral characteristics. Isoamyl alcohol (banana, fruity, solvent), isobutanol (baked, pharmacy, solvent), 1-propanol (alcoholic, sweet), and 1-butanol (alcoholic, banana) were detected in the order of highest amounts (Table 4). Yeast produces higher alcohols as by-products through reactions involving wort FAN (Stewart, 2017; Walker and Hill, 2016). The concentrations of isoamyl alcohol and isobutanol increased with longer germination or higher curing temperatures (Figure 7A and B). Isoamyl alcohol is specifically synthesized through the deamination process known as the Ehrlich pathway of leucine (Stewart, 2017; Kłosowski *et al*., 2015). Isobutanol is generated via the Ehrlich pathway of valine (Wess, Brinek and Boles, 2019). This is speculated to be a result of increased proteolytic enzyme activity due to prolonged germination, leading to elevated FAN content in tritordeum malt. Especially, the

highest concentrations were observed in G96h and K100°C for isoamyl alcohol (93.1 mg/L) and isobutanol (44.0 mg/L), respectively (Table 4). This is expected to be due to the limited Maillard reaction caused by insufficient sugar content following the short germination period. Also, leucine and valine are substances that are relatively less involved in the Maillard reaction (Ajandouz and Puigserver, 1999) so are expected to be absorbed by yeast. 1-propanol showed its highest concentration in G120h, with a decline as curing temperature increased. 1-propanol is formed through the metabolism of threonine during the fermentation process (Kelly, O'Connor and Kilcawley, 2023; Stewart, 2017). Threonine is recognized as a substrate consumed significantly in the Maillard reaction and known to generate furans that yeast cannot assimilate (Limacher *et al*., 2008; Ajandouz and Puigserver, 1999). The decrease in 1-propanol content at elevated temperatures could be attributed to the reduction of threonine due to the Maillard reaction.

Esters are the major congeners for aroma of whisky. Ethyl acetate (solvent-like, acetone, fruity) is the most predominant congener produced by yeast, and isoamyl acetate (banana, apple), ethyl hexanoate (apple, pear), and ethyl octanoate (butter cream) also play an important role (Stewart, 2017). Esters are generated through the enzymatic interaction between acetyl-CoA and higher alcohols (Dzialo *et al*., 2017). Low wine from tritordeum malts showed a higher content of ethyl acetate (6.7-11.4mg/L) compared to malted barley (6.5±0.6mg/L) (Table 4). Ethyl acetate has high thresholds (14-100 mg/L) (Lee *et al*., 2001), and at lower concentrations it imparts a fruity (apple-like astringent) aroma (Stanzer *et al*., 2023; Matias-Guiu *et al*., 2018). The model graph predicted an increase in ethyl acetate concentration with longer germination time and lower curing temperature (Figure 7D). Isoamyl acetate was detected in both tritordeum and barley low wines at levels of 0.1-0.2mg/L (Table 4). The response model indicated that isoamyl acetate would likely be detected to a slightly higher with prolonged germination (Figure 7E). It is noteworthy that ethyl hexanoate and ethyl lactate were not detected in low wine from barley malt but were detected in one sample each of tritordeum malt (Table 4).

Phenolic compounds in the whisky provide smoky, clove-like, and spicy aromas (Jeleń, Majcher and Szwengiel, 2019) and are mostly known as a characteristic of peated malt. Tritordeum contains various phenolic compounds, including ferulic and p-cinnamic acids (Suchowilska *et al*., 2021b; Montesano *et al*., 2020). Phenol (phenolic aroma) was detected most prominently in both tritordeum (110-153µg/L) and barley (182±2µg/L) (Table 4). As the germination period prolonged, it was anticipated that the phenol concentration would rise at temperatures below 80°C, however, above 80°C, a decrease in phenol content was expected (Figure 7F). The highest value (153.0µg/L) was observed at G96h and K100°C. It may be due to increased phenol content due to hemicelluloses hydrolysis at higher temperatures in the absence of adequate degradation of cell walls in grains with short germination (Călinoiu and Vodnar, 2019; Wang, He and Chen, 2014).

Even 4-vinyl guaiacol (clove-like, spicy) was only detected in six samples (172-287µg/L), the concentration detected was higher than in barley (120±104µg/L) (Table 4). Like wheat, ferulic acids are abundant in tritordeum, and 4-vinyl guaiacol is mainly found in beers made from wheat malt (Coghe *et al*., 2004). Ferulic acids are released during malting and the initial mashing stages, and hydrolysed during fermentation leading to elevated levels of 4-vinyl guaiacol, facilitated by the activity of the ferulic acid decarboxylase enzyme in yeast (Dzialo *et al*., 2017; Coghe *et al*., 2004; Lee *et al*., 2001). Further investigation is needed regarding those samples not detected. 4-ethyl guaiacol (smoked meat) was detected at higher levels in tritordeum (31.1-54.12µg/L) than in the barley (27.8±3.9µg/L) (Table 4). 4-ethyl guaiacol can be produced by the reduction of 4-vinyl guaiacol

through the action of vinylphenol reductase (Dzialo *et al*., 2017) or when ferulic acids are broken down by heat (Belitz *et al*., 2009). It is assumed that this is due to more ferulic acids contained in tritordeum. In the model graph, its concentration increased with longer germination periods and higher curing temperatures, which is predicted that the longer the germination period, the more ferulic acids were released and the concentration increased by heat (Figure 7G). 4-ethyl phenol (leather-like) was detected in two samples (6.2µg/L and 6.7µg/L), which also showed a higher concentration than barley (1.6±2.8µg/L) (Table 4).

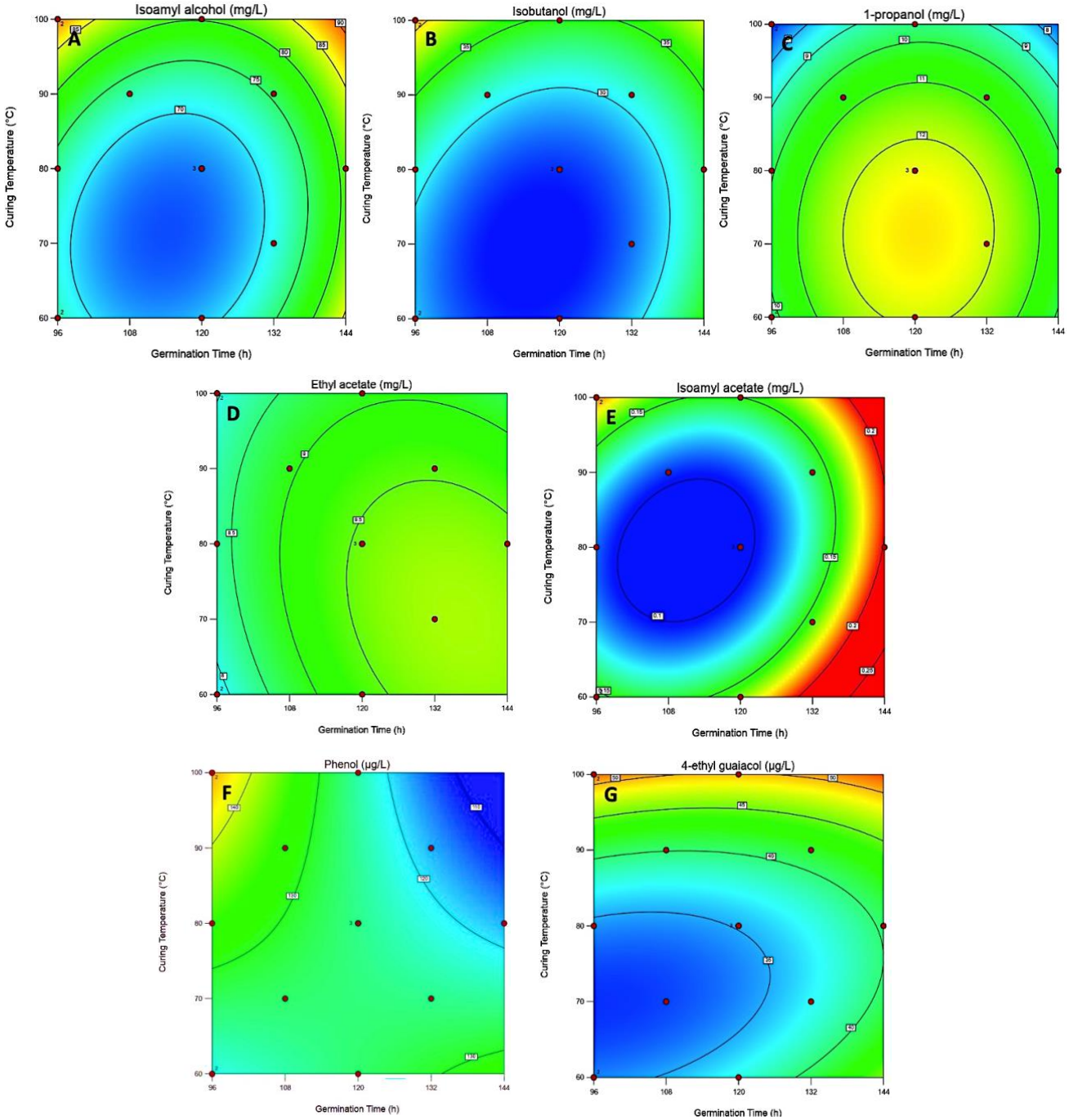


Figure 7. Concentration of congers in low wine from tritordeum malts. (A) isoamyl alcohol (mg/L); (B) Isobutanol (mg/L); (C) 1-propanol (mg/L); (D) Ethyl acetate (mg/L); (E) Isoamyl acetate (mg/L); (F) Phenol (µg/L); (G) 4-ethyl guaiacol (µg/L). As the colour goes from blue to red, it indicates the value increases. The red dots indicate data points (5 points missing for higher alcohols and esters; 4 points missing for phenolic compounds).

4. Conclusion

This study confirmed that germination time and curing temperature during the malting process influence the characteristics and quality of tritordeum malt, and that variations in the malting process distinctly impact the aroma profile of low wine volatile compounds of tritordeum. Furthermore, it was also shown that low wine of tritordeum has different congener profiles and concentrations compared to malted barley. Although a few data points were missed in the response surface model due to low wort recovery rates, the model graph predicted intriguing outcomes. Particularly, it is noteworthy and important that the parts of the model where data point coverage was higher exhibited significant trends. The modeling predicts that optimal tritordeum malt characteristics and high gravity wort quality for distillation can be achieved with a longer germination period and curing at around 80°C. Despite instances of low wort volume in some malting regimes, most samples were produced without filtration issues even in the absence of husks. In addition, distiller yeast exhibited comparable fermentation efficiency to in the wort of tritordeum malt to barley, resulting in the production of similar or higher alcohol yield of low wine. Daute *et al*. (2023) demonstrated that the production phase of low wine offers the potential for preselecting aromas and flavours. Identifying volatile compounds in tritordeum low wine would play a crucial role in predicting the aroma and indicating the guideline for the production of new make spirits. It is anticipated that low wine made by tritordeum malt with its distinct congeners and concentrations compared to malted barley, will be capable of producing new make spirits with novel aromas to introduce in the industry.

Acknowledgments

I would like to express my deepest gratitude to my supervisor, Dr. Calum Holmes, for his invaluable guidance, assistance, and support throughout this study. I am thankful to Cristal Peck from Boortmalt for her advice on tritordeum malt and Etienne from Vivagran S.L for providing the grain. Additionally, I acknowledge Maarten Gorseling and Alasdair Brown, whose help has been instrumental in various aspects, also Takehiko Hiura and Alan Philip for their kind advice. I appreciate the Worshipful Company of Distillers for providing a scholarship to allow extensive analysis of compounds. Lastly, my heartfelt thanks to my family who supported me to study.

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