Studii şi Cercetări Ştiinţifice
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară
2025, **26** (2), pp. 239 – 255
https://doi.org/10.29081/ChIBA.2025.644
Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry
ISSN 1582-540X

ORIGINAL RESEARCH PAPER

USE OF WHITE, RED, AND BLACK QUINOA IN UNCONVENTIONAL BREWING MASH MATRICES - A TECHNOLOGICAL APPROACH

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Received: June, 14, 2025
Accepted: June, 24, 2025

Abstract: The growing demand for gluten-free, low- and non-alcoholic beer, has led to increased research into the incorporation of non-traditional cereal substrates in the brewing process, such as the pseudocereal quinoa (*Chenopodium quinoa*, Willd.). This study investigates the brewing potential of three quinoa varieties - white, red, and black - as adjuncts for the development of health-oriented and diversifying beer assortments. Three unconventional quinoa-based mashes were formulated using Pilsner barley malt, quinoa varieties, and acid barley malt at proportions of 54:40:6 % ($w \cdot w^{-1}$). The ratio of cereal ingredients to mashing water was 1:4.5. The mashing protocol involved a stepwise temperature increase of 1 °C·min^{-1}, with rests at 45 °C for 30 minutes, 63 °C for 20 minutes, 72 °C for 30 minutes, and 78 °C for 10 minutes. Boiled malt worts with Amarillo hop pellets for 30BU were fermented with lager and top yeasts culture. The mashing, lautering, and fermentation performance of the purpose quinoa-based brewing matrices were briefly monitored according to the *Analytica-EBC* methods. The results demonstrated that the studied quinoa varieties could represent alternative substrates to conventional brewing mashes and revealed distinct technological characteristics and varying brewing behaviors among the quinoa varieties, indicating that each variety may influence the brewing process differently.

Keywords: *brewing, health-promoting potential, hop, malt, mashing, specialty beer, wort, yeast*

INTRODUCTION

Recent statistics show that beer production is steadily increasing both globally and within Europe. In 2023, global beer output reached approximately 1.88 billion hectolitres, reflecting sustained demand and market resilience [1, 2]. At the same time, brewers are actively diversifying their product lines to align with evolving consumer expectations, particularly through the use of alternative raw materials [3 – 9]. Among these, pseudocereals such as quinoa (*Chenopodium quinoa* Willd.) have gained notable attention due to their gluten-free nature, functional properties, and high nutritional value. Scientific studies report that substituting up to 30 - 40 % of barley malt with quinoa can be achieved without compromising wort quality, while even enhancing foam stability and the overall nutritional and sensorial profile of the final product [3, 4, 10 – 15].

During the last few years, the global interest in quinoa (*Chenopodium quinoa* Willd.) has grown significantly, driven by both its agronomic versatility and exceptional nutritional profile [7, 16 – 19].

Quinoa has been commonly prepared in cooked dishes such as salads, porridges, soups, stews, and fried patties [13, 20 – 22]; however, it is increasingly being used in the form of "healthy" snack products, granola bars, breads, pasta and beverages, with more recent applications extending to beer [4, 11, 19, 22 – 27]. Given its rich biochemical composition and increasing global availability, quinoa presents promising potential for applications beyond traditional food uses, including in the brewing industry. The exploration of quinoa as a partial substitute for malted barley in beer production is of particular interest in the context of developing innovative, nutritionally enriched, or gluten-reduced beer products [5, 12, 28].

Quinoa has emerged as a promising ingredient in unconventional brewing matrices due to its exceptional nutritional and functional properties [17, 29]. Quinoa's high nutrient content, including minerals, dietary fibers, vitamins, essential amino acids, proteins, polyunsaturated lipids, and antioxidants, contributes to the enhancement of beer's nutritional profile [15, 20, 23, 30, 31]. Recent studies have explored the use of quinoa in brewing, with a focus on developing gluten-free [8, 9, 32, 33] and low-alcohol beers [12], not only due to its nutritional benefits but also because it contributes to the sensory profile of the beer by introducing distinctive flavors, aromas, and color nuances specific to each quinoa variety, white, red, and black. In brewing applications, quinoa can be used as a partial replacement for barley malt, significantly increasing the content of essential metal ions, including calcium, iron, zinc and magnesium, in the wort, which are crucial for yeast performance and fermentation efficiency [6, 31]. The concentrations of these are significantly higher than those found in most conventional cereal grains [28, 31]. Previous studies have reported that the inclusion of 10% quinoa as a substitute for barley malt may increase the content of zinc and magnesium ions by 41 % and 49 %, respectively, and indeed enhancing the nutritional value of the beer wort, and the brewer's yeast management was improved, including the fermentative capacity of brewing yeast [31].

Additionally, the use of quinoa in brewing can lead to technological and processing benefits, for example, fermentation of quinoa-based wort can reduce anti-nutritional factors like phytates, which inhibit mineral absorption [12, 34 – 36]. This process enhances the bioavailability of essential minerals, further improving the nutritional

profile of the brewed product [15, 21, 32]. However, the serial repitching of yeast in quinoa wort fermentations has shown limitations, with a general weakening of yeast performance observed after several successive fermentations [3, 28, 32, 34, 35]. Despite these challenges, quinoa-based beers have demonstrated improved foam stability, and sensory qualities (favorable and distinct profile of flavor compounds synthesized by yeast from amino acids and fatty acids derived from quinoa), offering brewers the opportunity to create unique and appealing products [4, 5, 12, 14, 28, 32, 34, 35].

Quinoa is a rich source of bioactive compounds, including flavonoids, phenolic acids, carotenoids, and saponins, which have been associated with anti-inflammatory, antioxidant, and cancer-preventive effects [10, 15, 36, 37].

Additionally, quinoa provides important vitamins including C, E, and folic acid, supporting its classification as a functional food. These constituents may enhance the health-promoting potential of the resulting beer, potentially offering protective effects against various chronic diseases [6, 10, 38]. The incorporation of quinoa as a brewing adjunct not only caters to the growing demand for gluten-free and nutritious beverages but also aligns with sustainable food production practices [4, 8, 9].

Based on the considerations outlined above, the research on quinoa-based brewing matrices is both timely and relevant. This study aimed to conduct a comparative technological evaluation of three unmalted quinoa varieties (white, red, and black), used as 40 % substitutes for barley malt in mashing. To adjust the *p*H of the mashes and the resulting worts, 6 % (w/w) acidulated barley malt was included in the quinoa-based formulations. Additionally, to enable a proper interpretation of the results, two control mashes were prepared: one composed solely of *Pilsner* malt, and the other consisting of Pilsner malt combined with 6 %($w \cdot w^{-1}$) acidulated malt. *Amarillo* hops pellets, used as a single-hop variety for boiling, were selected for their capacity to impart both bitterness and a complex aromatic profile; aromatic beer styles often incorporate this hop variety. The research was further extended by assessing the fermentative performance of the quinoa-based worts using two yeast strains: one for bottom fermentation (lager) and one for top fermentation (ale).

The technological behavior of the quinoa-based brewing matrices was assessed through selected indicators related to mashing, filtration efficiency, and fermentation capacity, following *Analytica-EBC* methods [39].

The results of this study underscore quinoa's technological potential as a sustainable ingredient in specialty brewing. Moreover, among the three quinoa varieties, white quinoa demonstrated the greatest technological efficiency as a substitute for *Pilsner* malt. However, further research and technological innovation are required to optimize the use of these different quinoa varieties and to address challenges related to mash composition, wort and beer chemistry, sensory attributes, and the performance of specific yeast strain.

MATERIALS AND METODS

Materials

Raw materials

Pilsner barley malt (*PM*) [40] and acidulated barley malt (*AM*) [41] were procured from Weyermann® Specialty Malting, Bamberg, Germany, through S.C. BRICO IDEEA S.R.L. București/Romania. Quinoa (*Chenopodium quinoa*, Wild.), in red (*RQ*), white (*WQ*) and black (*BQ*) varieties, was acquired from Driedfruits® Supplier, located in Râmnicu Vâlcea, Romania, with Peru as the country of origin. Their main characteristics were as follows:

WQ: moisture - 13.5 % ($w \cdot w^{-1}$); total carbohydrates - 67.5 % ($w \cdot w^{-1}$), of which 5.0 % were sugars; proteins - 13.6 % ($w \cdot w^{-1}$); lipids - 4.5 % ($w \cdot w^{-1}$); total fibers - 5 % ($w \cdot w^{-1}$).
RQ: moisture - 13.4% ($w \cdot w^{-1}$); total carbohydrates - 57.2 % ($w \cdot w^{-1}$), of which 4.9 % were sugars; proteins - 14.1 % ($w \cdot w^{-1}$); lipids - 6.1 % ($w \cdot w^{-1}$); total fibers - 7 % ($w \cdot w^{-1}$).
BQ: moisture - 13.2 % ($w \cdot w^{-1}$); total carbohydrates - 64.4 % ($w \cdot w^{-1}$), of which 4.4 % were sugars; proteins-15.6 % ($w \cdot w^{-1}$); lipids- 4.4 % ($w \cdot w^{-1}$); total fibers-13.3 % ($w \cdot w^{-1}$).
A visual presentation of the ingredients is provided in Figure 1.

(PM) *(AM)* *(WQ)* *(RQ)* *(BQ)*

Figure 1. *Photographic image of the raw materials grain utilized in the brewing trials (PM - Pilsner barley malt; AM - acidulated barley malt; WQ - white quinoa; RQ - red quinoa; BQ - black quinoa)*

Water

Samples were prepared using distilled water produced by standard laboratory distillation equipment from Boeco, Germany.

Hop pellets

The worts were boiled with Amarillo hop pellets (8.5 % α-acids, 6.2 % β-acids; country of origin: USA), which were purchased from S.C. BRICO IDEEA S.R.L., Bucharest, Romania - a distributor of hop products from Brouwland, Belgium. These hops are known for their dual-purpose use, contributing to both moderate bitterness and a distinctive citrus-floral aroma.

Yeast strains

Fermentation of worts was carried out with two commercial active dry brewer's yeast (*ADY*) from Brouwland, Belgium: Brewferm® Top (a top-fermenting strains, *Saccharomyces cerevisiae*), with an optimal fermentation range of 18-25 °C, attenuation of ~72%, and a fermentation profile characterized by moderate ester formation and a mildly fruity aroma; and Brewferm® Lager (a bottom-fermenting strains,

Saccharomyces pastorianus), with an optimal range of 10 - 15 °C, attenuation of ~78 %, and a clean fermentation profile with minimal off-flavors.

Methods

Milling regime

All raw materials were ground using a Universal Laboratory Disc Mill, Type DLFU (Bühler AG, Switzerland), which was set for fine milling with a 0.2 mm disk gap. The obtained whole grists flour (Figure 2) were analyzed for particle size distribution using a Retsch's Laboratory Vibratory Sieve Shaker, AS 200 basic, operated at an amplitude of 2.5 mm for 10 minutes.

(a) *(b)* *(c)* *(d)* *(e)*

Figure 2. *Photographic image of the whole grists utilized in the mashing trials corresponding to the raw materials: (a)-PM grist*; (b)-*AM grist*; (c)-*WQ grist*; (d)-*RQ grist*; (e)-*BQ grist*

Mashing regime

The grists were mixed with water at a proportion of 1:4.5 and mashing process was conducted using 1-Cube Mashing Bath (type R4), Czech Republic, following the mashing diagram presented in Figure 3.

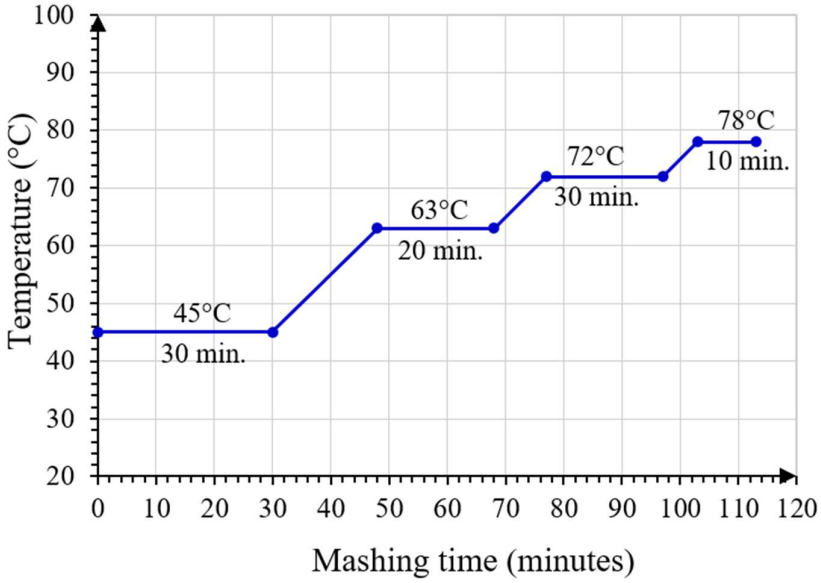

Figure 3. *Mashing program for quinoa-based brewing matrices with gradual increase of the temperature (1 °C·min^{-1})*

The grist mash formulations and their corresponding codifications used in this study are presented in Table 1. To evaluate the brewing potential of the three quinoa varieties as adjuncts, two control mashes were prepared: one with 100 % Pilsner malt, and another with Pilsner malt and 6 % acidulated malt - the same acidulated malt ratio used in the experimental quinoa mash formulations.

Table 1. *Grist mash formulations and codification of resulting mash variants*

Mash ingredient	Code of mashing variants				
	M1	***M2***	***M3***	***M4***	***M5***
Pilsner barley Malt (*PM*) [%(w·w^{-1})]	54	54	54	94	100
Acidulated barley Malt (*AM*) [%(w·w^{-1})]	6	6	6	6	-
White Quinoa (*WQ*) [%(w·w^{-1})]	40	-	-	-	-
Red Quinoa (*RQ* [%(w·w^{-1})]	-	40	-	-	-
Black Quinoa (*BQ*) [%(w·w^{-1})]	-	-	40	-	-

The mash was continuously homogenized at a speed of 100 rpm with a temperature increase of 1 °C·min^{-1}. According to the *Analytica-EBC* Methods [39] were performed: - saccharification test (10 minutes after the mash temperature reaches 72 °C, the determination of the saccharification time begins, by applying a drop of *Lugol*'s iodine solution to a small sample of the mash; the absence of a blue or purple color confirm complete saccharification), *p*H, extract, and color of wort samples collected after lautering.

Lautering (Mash filtration) regime

The saccharified mashes were lautered following EBC method 4.5.1, using fluted filter paper (No. 597 ½, 320 mm diameter, from *Whatmann*), into graduated cylinders (500 mL) and the amount of wort was recorded at 1-minute intervals for the first 10 minutes, and subsequently at 5-minute intervals for a total duration of 105 minutes, and beyond this point if required. The initial 150 mL portion of the filtered malt wort was recirculated over the filter paper

The technological potential of the three quinoa varieties was also evaluated by determining the soluble extract yield of the brewer's spent grain (*BSG*) obtained from the filtration of the saccharified mashes *M1*, *M2*, and *M3*.

For this 25 g of fresh *BSG* sample was collected immediately after malt wort separation and mashing with 275 mL water at 200 rpm for 1 hour at 78 °C. Then the *BSG*-mash was cooled to 20 °C, adjusted to the original dilution, and filtered in a manner similar to the primary mash, in order to collect the weak wort, equivalent to the running wort.

The formula used to calculate the soluble extract yield of all *BSG* samples obtained in the present research is as follows:

$$R_{BSGi} = \frac{E_{BSGi} \cdot (1100 + H_{BSGi})}{100 - E_{MSGi}} \quad (1)$$

$$R'_{BSGi} = \frac{R_{BSGi}}{100 - H_{BSGi})} \cdot 100 \quad (2)$$

where:

R_{BSGi} is the soluble extract yield of the *BSGi* at matter as is for M_i ($i = \overline{1,5}$) mashing variants, (%);

R'_{BSGi} is the soluble extract yield reported at dry matter for M_i $(i = \overline{1,5})$ mashing variants, (%),
E_{BSGi} is the extract of the weak wort, which corresponds to the soluble fraction recovered from the BSG_i, $(i = \overline{1,5})$, (°P),
H_{BSGi} is the moisture content of the *BSGi* for M_i $(i = \overline{1,5})$ mashing variants, %.

Wort boiling regime

Malt wort boiling (60 minutes) was carried out under laboratory conditions using a 1 L round-bottom flask placed in a controlled heating mantle (Electrothermal™, EM1000 Series, Electrothermal, UK). The heating system provided uniform heat distribution throughout the process. To ensure constant volume boiling and minimize evaporative losses, a vertical reflux condenser with an internal cooling coil (connected to the laboratory tap water supply) was mounted on the flask. A single dose hop addition was calculated with formula (1) to achieve 30 IBU (Bitterness Units) and was introduced into the flask together with the malt wort at the start of the boil, prior to the installation of the condenser.

$$D_{AH} = \frac{10 \cdot IBU}{\alpha AA \cdot \eta_{isom.}} \quad (3)$$

where:
D_{AH} is hop pellet dose, ($g \cdot L^{-1}$),
IBU is International Bitterness Units, ($mg \cdot L^{-1}$),
αAA is the alpha acid content of the hop pellets, (%, $w \cdot w^{-1}$),
$\eta_{isom.}$ is the isomerization yield of alpha acids, (%, $w \cdot w^{-1}$).
After boiling, the wort was cooled and clarified by filtration using the same grade of filter paper as that employed for the filtration of the saccharified mash.

Fermentation regime

Fermentation of the filtered boiled wort samples was carried out to determine their limit of apparent attenuation (apparent final degree of fermentation) (*AAL)*, in accordance with the *Analytica-EBC Methods* [39], and expressed as a percentage. The amount of *ADY*s (*Lager* (*LY*) and *Top* (*TY*) yeast strains) used for inoculating the worts was calculated based on the standard method, which employs 15 g of yeast (containing 20 % d.m.) per 200 mL of wort. The determination was carried out at 25 °C under continuous stirring for 24 hours. The calculation formula for LAA is presented below (Equation 4):

$$LAA = \frac{E_i - E_{af}}{E_i} \cdot 100 \quad (4)$$

where:
AAL is apparent attenuation limit of beer wort (%),
E_i is the initial extract of the beer wort, that is, before fermentation (°P; degree *Plato*),
E_f is the apparent final extract of the beer wort, that is, before fermentation (°P).

Moisture content (*H*, %) of raw materials, hop pellets, brewers' spent grain, and brewer's yeast was determined according to the *Analytica-EBC Methods* [39], to ensure accurate ingredient dosing and to enable precise calculation of extraction yields based on the experimental data. Wort extract was determined according to *Analytica-EBC*

Methods [39] using a portable digital densitometer (DMA 35, from *Anton Paar*, Austria). The viscosity of the worts, expressed in mPa·s, was determined at 20 °C using the *Ubbelohde* glass capillary viscometer, according to Method 8.4 of *Analytica-EBC*.
The calculation of extract yield (*R*, %), and extract yield reported at dry matter (*R'*, %) of the analyzed mashes in this study are presented and detailed below:

$$R_i = \frac{E_{Mi} \cdot (450 + 0.06 \cdot H_{AP} + 0.40 \cdot H_Q + 0.54 \cdot H_{PM})}{100 - E_{Mi}} \quad (5)$$

$$R_i' = \frac{R_i}{100 - (0.06 \cdot H_{AP} + 0.40 \cdot H_Q + 0.54 \cdot H_{PM})} \cdot 100 \quad (6)$$

$$R_i = \frac{E_{Mi} \cdot (450 + 0.06 \cdot H_{AP} + 0.40 \cdot H_Q + 0.54 \cdot H_{PM})}{100 - E_{Mi}} \quad (7)$$

$$R_i' = \frac{R_i}{100 - (0.06 \cdot H_{AP} + 0.40 \cdot H_Q + 0.54 \cdot H_{PM})} \cdot 100 \quad (8)$$

where:
R_i is the extract yield reported at matter as is for M_i ($i = \overline{1,3}$) mashing variants, (%),
R_i' is the extract yield reported at dry matter for M_i ($i = \overline{1,3}$) mashing variants, (%),
H_{AP} is the moisture content of the *AM*, (%),
H_Q is the moisture content of the *QW*, *RQ*, respectively *BQ*, (%),
H_{PM} is the moisture content of the *PM*, (%).

The calculation formulas for *R* and *R'*, for the control mashes *M4* and *M5*, are as follows:

$$R_4 = \frac{E_{M4} \cdot (450 + 0.06 \cdot H_{AP} + 0.94 \cdot H_{PM})}{100 - E_{M4}} \quad (9)$$

$$R_4' = \frac{R_4}{100 - (0.06 \cdot H_{AP} + 0.94 \cdot H_{PM})} \cdot 100 \quad (10)$$

$$R_5 = \frac{E_{M5} \cdot (450 + H_{PM})}{100 - E_{M5}} \quad (11)$$

$$R_5' = \frac{R_5}{100 - H_{PM})} \cdot 100 \quad (12)$$

Statistical analysis
For data analysis was applied MATLAB R2023b version software for One-way analysis of variance (ANOVA). The determination of significant differences on measured values was used the Tukey test set at $p \leq 0.05$. All the samples were made in triplicate (n=3) and the experimental results are noted in the tables as means followed by standard deviation.

RESULTS AND DISCUSSION

As a result of the moisture content analysis conducted on the malt and quinoa samples, the following results were obtained: H_{PM} = 4.3 %, H_{AP} = 6.7 %, H_{WQ} = 12.6 %,

H_{RQ} = 13.5 %, and H_{BQ} = 12.9 %. Additionally, the active ADY strains *LY* and *TY* showed moisture contents of 6.4 % and y %, respectively 5.3. The Amarillo hop pellets exhibited a moisture level of 8.6 %.

Particle Size Distribution of Brewing Raw Materials

The particle size distribution analysis (Table 2) revealed significant differences ($p < 0.05$) between the grist fractions of *PM*, *AM*, and the three quinoa varieties - *WQ*, *RQ*, and *BQ*.
These differences highlight the impact of grain structure and mechanical properties on milling behavior.

Table 2. *The particle size distribution of the Pilsner barley malt, acidulated barley malt, Red Quinoa, White Quinoa and Black Quinoa*

Number of sieve (separated fractions' name)	**Mesh width [mm]**	**Separated fractions on sieve [%m/m]**				
		***Pilsner* barley malt** (*PM*)	**Acidulated barley malt** (*AM*)	**Quinoa**		
				White (*WQ*)	**Red** (*RQ*)	**Black** (*BQ*)
1 (Husks)	1.250	2.16 ± 0.05	(9 ± 0.19)·f	(2 ± 0.12)·f	(5 ± 0.10)·f	0
2 (Coarse grists I)	0.630	16.16 ± 0.29	15.36 ± 0.31	22.53 ± 0.74	41.27 ± 0.84	37.31 ± 0.85
3 (Coarse grists II)	0.400	29.44 ± 0.56	35.12 ± 0.90	39.65 ± 0.77	33.45 ± 0.86	36.59 ± 0.76
4 (Fine grists I)	0.315	12.67 ± 0.25	14.60 ± 0.29	12.30 ± 0.17	8.84 ± 0.17	10.88 ± 0.20
5 (Fine grists II)	0.250	7.43 ± 0.14	13.34 ± 0.32	7.95 ± 0.14	5.43 ± 0.13	5.36 ± 0.14
6 (Fine grists III)	0.160	7.72 ± 0.19	10.70 ± 0.22	9.60 ± 0.13	6.37 ± 0.13	5.55 ± 0.11
Bottom (Flour)	-	24.42 ± 0.63	10.87 ± 0.28	7.96 ± 0.10	4.63 ± 0.12	4.30 ± 0.09

Values represent the mean ± standard deviation (n = 3). All values are statistically different at $p < 0.05$, based on posthoc Tukey method. Notation: f-multiplication factor, $f = 10^{-3}$.

In the case of traditional brewing malts, *PM* exhibited the highest percentage of flour (24.42 ± 0.63 %), followed by coarse grist II (29.44 ± 0.56 %), indicating an effective fragmentation pattern suitable for enzymatic access during mashing. *AM*, by contrast, showed a reduced flour fraction (10.87 ± 0.28 %), but a higher proportion of coarse and fine grists, particularly coarse grist II (35.12 ± 0.90 %) and fine grist II (13.34 ± 0.32 %), suggesting a denser granular matrix, possibly due to the special technological process applied to obtain this type of malt.
Nevertheless, the particle size distribution of *AM* does not appear to significantly affect lautering performance with respect to the structure of the filter bed. This minimal impact can be attributed to the relatively low inclusion rate of *AM* - only 6 %($w \cdot w^{-1}$) of the grist subjected to mashing, which is insufficient to considerably modify the filter bed structure. The primary effect of *AM* is instead manifested through its contribution to the modulation of solubilization conditions and enzymatic hydrolysis during the mashing process.
The quinoa samples displayed markedly different behavior. *WQ* demonstrated a more balanced distribution, with a prominent proportion in the coarse grist II fraction (39.65 ± 0.77 %), accompanied by moderate amounts in both flour (7.96 ± 0.10 %) and fine grists. *RQ* showed a strong concentration in the coarser fraction (41.27 ± 0.84 % in coarse grist I) and lower flour content (4.63 ± 0.12 %), reflecting a higher resistance to

fine milling. Similarly, *BQ* had a high proportion in coarse grist I (37.31 ± 0.85 %), while its flour yield was the lowest among all samples (4.30 ± 0.09 %).
These findings suggest that the quinoa varieties exhibit less friability compared to barley malts, resulting in coarser milling profiles. This could have implications for mash filtration efficiency and enzymatic accessibility.
Among the quinoa types, *WQ* appears to offer the most suitable granulation profile for brewing purposes, due to its intermediate distribution across fine and coarse grist fractions and relatively higher flour yield.

Mashing performance

A preliminary assessment of the mashing process was conducted through the determination of saccharification time, defined as the interval starting from the point at which the mash reached a temperature of 72 °C. The corresponding results are presented in Table 3 and indicate that replacing 40 % of *Pilsner* malt with quinoa, in combination with the addition of 6 % acidulated malt, is technologically feasible even in the absence of exogenous enzyme supplementation. These results are consistent with and supported by those reported in previous studies [4, 11, 31]. The longest saccharification time was observed for the mash containing WQ, which can be attributed to its higher total carbohydrate content (67.5 % ($w \cdot w^{-1}$)), as well as to the lower initial *p*H (4.92 ± 0.01) recorded at the onset of mashing. Table 4 presents data on the *p*H evolution of the mashes during the mashing process, along with the *p*H values of the wort following both boiling and fermentation. The data reflect dynamic changes in *p*H across mashing (after 10 minutes), wort preparation (after lautering and boiling), and subsequent alcoholic fermentation using two yeast strains: a bottom-fermenting (lager) strain and a top-fermenting (ale) strain.

Table 3. *Saccharification time of mashes considered for the study*

Characteristics	Code of mashing variants				
	M1	***M2***	***M3***	***M4***	***M5***
Saccharification time [minutes]	20-25	20	20	15	10 - 15

As shown in Table 4, the wort obtained from mash *M1* exhibits a *p*H value closely aligned with that of the wort derived from mash *M5* (100 % Pilsner malt), suggesting that the use of *WQ* as a brewing adjunct may be the most appropriate, despite its initially lower mash *p*H. The *p*H value observed for sample *M4*, along with the data from all three quinoa varieties, indicates that substituting malt with quinoa tends to increase mash *p*H. Consequently, the addition of acidulated malt (*AM*) becomes necessary (in this case, 6 % ($w \cdot w^{-1}$), a proportion selected based on the technical specifications provided for this specialty malt.

Table 4. *pH changes in the evaluation process of quinoa-based mashes*

Mashing code	*p*H				
	***After 10 minutes of* mashing**	**Wort *after lautering***	**Wort *after boiling***	**After fermentation with**	
				Lager yeast strain	***Top*** yeast strain
M1	4.92 ± 0.01	5.58 ± 0.03	5.41 ± 0.03	3.95 ± 0.01	4.31 ± 0.02
M2	5.04 ± 0.02	5.57 ±0.02	5.48 ± 0.02	4.09 ± 0.02	4.37 ± 0.04
M3	5.06 ± 0.03	5.55 ±0.03	5.40 ± 0.03	4.11 ± 0.01	4.38 ± 0.03
M4	4.58 ± 0.03	5.16 ±0.02	5.03 ± 0.02	3,96 ± 0.02	4.15 ± 0.01
M5	5.22 ±0.02	5.63 ±0.03	5.44 ± 0.04	4.03 ± 0.01	4.18 ± 0.02

Values represent the mean ± standard deviation (n=3). All values are statistically different at $p<0.05$, based on posthoc Tukey method.

It is important to note that the mash formulations used in this study (see Table 1) are preliminary, intended to establish technological feasibility limits for incorporating quinoa in brewing. Even after wort boiling with hops, the resulting *p*H values remained within acceptable technological ranges, indicating readiness for subsequent fermentation.

Following fermentation with the *LY* yeast strain, the final *p*H values were lower (3.95 - 4.11) compared to those obtained using the *TY* strain, which produced slightly higher *p*H levels (4.15 - 4.38). This trend was consistent across all mash formulations and reflects the distinct metabolic profiles of the yeast strains employed.

Evidence supporting the potential of quinoa as an alternative starch source in brewing is also reflected in the data presented in Table 5.

Table 5. *Characterization of the mashing formula by determining wort extract, color, and extract yield relative to dry matter for mashes and associated BSGs*

Characteristics	Code of mashing variants				
	M1	***M2***	***M3***	***M4***	***M5***
Wort extract, E [°P]	14.8 ± 0.11	14.2 ± 0.09	13.7 ±0.10	15.1 ±0.13	15.2 ± 0.14
Wort color, C [EBC]	5.0 ± 0.06	4.7 ± 0.04	5.1 ± 0.04	3.7 ± 0.06	4.1 ± 0.03
Extract yield, *R'* [%]	86.21 ± 0.27	82.52 ± 0.38	78.91 ± 0.43	84.58 ± 0.46	85.09 ±0.54
Soluble extract yield of *BSG*, R'_{BSG} [%]	60.28 ± 0.42	56.42 ± 0.54	49.64 ± 0.45	72.39 ± 0.64	52,17 ± 0.44

Values represent the mean ± standard deviation (n=3). All values are statistically different at $p<0.05$, based on posthoc Tukey method.

The extract of the worts obtained after lautering are relatively close to those produced by the control mash *M5* (100 % *PM*), suggesting that quinoa can contribute effectively to wort fermentability. Moreover, the color measurements of the quinoa-based worts fall within the typical range for pale beers, despite being slightly higher than that of the control sample. This slight increase in color intensity is likely due to the presence of acidulated malt in the formulation, which is known to influence both mash *p*H and color characteristics due to its specific production process.

Based on the values of *R'* and R'_{BSG}, it can be observed that *WQ* exhibits the highest technological utilization efficiency, followed by BQ. Furthermore, by applying equations (5) to (12) described in the *Methods* subsection, the R′ extract yields for the

three quinoa varieties were calculated as follows: R'_{WQ}=87.49 ± 0.42 %, R'_{RQ}=78.27 ± 0.31 %, and R'_{BQ}=69.24 ± 0.28 %.
Although taste and aroma characteristics of these worts were evaluated during the course of this study, the corresponding results are not included in the present article. This decision was based on the extensive existing literature, which consistently reports the positive impact of quinoa addition on the sensory attributes of beer.
The results demonstrate that incorporating quinoa into the grist composition can maintain essential brewing quality parameters, making it a technologically suitable adjunct.

Lautering performance

Figure 4 presents the lautering performance of quinoa-containing mashes in comparison with the control variants, *M4* and *M5*. The *M5* control mash exhibited stable lautering behavior, with consistent flow rates and low filtration resistance - characteristic of mashes based predominantly on well-modified barley malt with sufficient husk material.

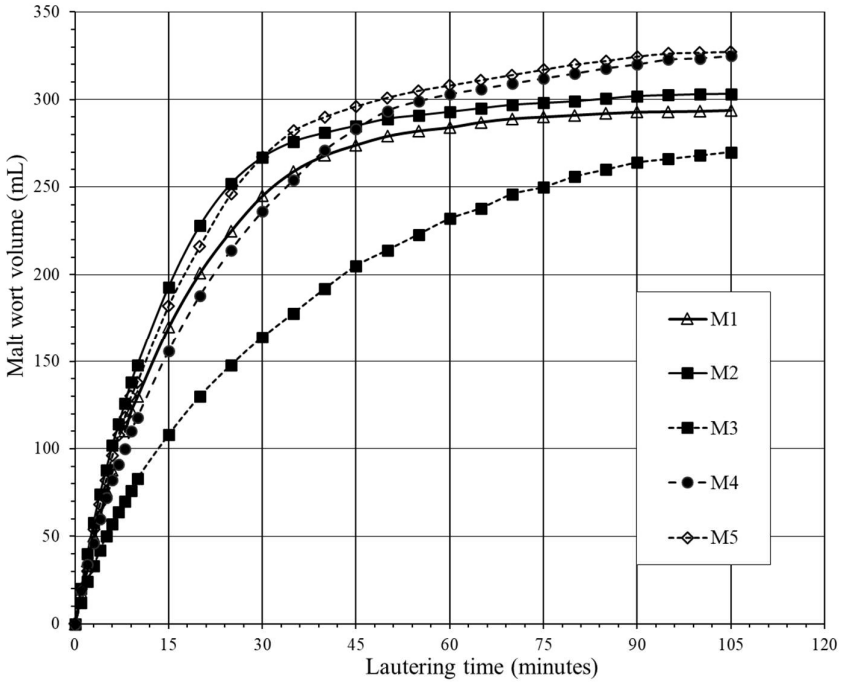

Figure 4. *Lautering performance of quinoa-based and control (M4 and M5) mashes*

The *M4* control mash showed that the addition of acidulated malt reduced wort filtration rate. Nonetheless, the collected wort volume was comparable to that of the *M5* mash. This may be due to the higher proportion of Coarse Grists II and Fine Grists I-III in the *AM* grist, relative to the *PM* grist, as wort viscosities were similar despite significantly different initial mash *p*H values (see Table 4).
Among the quinoa-based mashes, filtration performance differed notably, as reflected by lautering time and collected wort volume. *M2* exhibited the highest wort separation

rate - exceeding all the other mashes studied during the first 30 minutes - while *M3* showed the lowest. As shown in Figure 4, *M2* also yielded the highest wort volume, whereas *M3* yielded the lowest. These differences are likely attributable to the milling behavior of the three quinoa varieties, rather than to extract concentration, as the wort gravities (E_{M1} = 14.8 °P, E_{M2} = 14.2 °P, E_{M3} = 13.7 °P) do not explain the volume discrepancy. Moreover, the three worts had nearly identical mean viscosities: η_{M1} = 1.94 mPa·s, η_{M2} = 1.93 mPa·s, and ηM3 = 1.93 mPa·s.

The observed negative effect is directly attributable to the high quinoa substitution level (40 % of the grist), which represents the upper limit permitted by both brewing practice and legal regulations for adjunct usage in beer production. Additionally, no exogenous enzymatic preparations were used in this study.

As shown in Table 2, all quinoa variants were devoid of husk material and exhibited a significantly different particle size distribution. The quinoa grists were dominated by intermediate-sized fractions (0.400 - 0.630 mm), particularly in *RQ* (41.27 ± 0.84 % in Coarse Grist I), and contained less than 5 % flour, in contrast to *PM*, which had 24.42 ± 0.63 %.

The absence of husk, combined with the altered granulation profile, compromised filter bed structure and permeability. In conventional mashes, husk fragments contribute to bed porosity and mechanical stability, promoting effective wort runoff. At a 40 % substitution level, the structural contribution from residual malted barley was insufficient to compensate for quinoa's deficiencies, resulting in impaired lautering performance.

These findings underscore a key limitation in the use of quinoa at high inclusion rates. While quinoa may enhance the nutritional and sensory profile of beer, its incorporation at the maximum allowable threshold necessitates process adaptations, such as grist reformulation, the use of filtration aids, or adjustments to lautering protocols to maintain brewing efficiency.

Fermentation performance

The results obtained for the apparent attenuation limit (*AAL*) and potential alcohol concentration are presented in Figures 5 and 6, respectively. These data indicate that the hopped worts prepared with *WQ* (white quinoa) exhibit the highest fermentative potential. Additionally, the use of the Lager yeast strain (*LY*) led to higher degrees of fermentation across all wort samples when compared to the Top-fermenting yeast strain (*TY*).

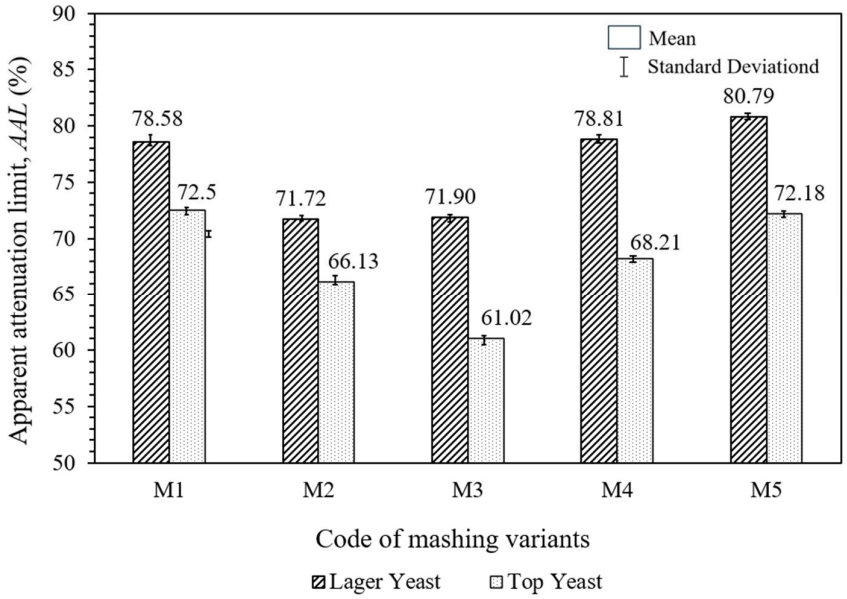

Figure 5. *Limit of apparent attenuation (apparent final degree of fermentation) of boiled wort samples*

Also, the experimental values illustrated in Figures 5 and 6 demonstrate that the complex composition of these worts [12, 28, 31, 32, 34, 35] compensates for their relatively lower extract concentrations. Despite this, the worts presented similar initial *p*H values at pitching. This compensatory effect is particularly evident in fermentations carried out with the *LY* strain.

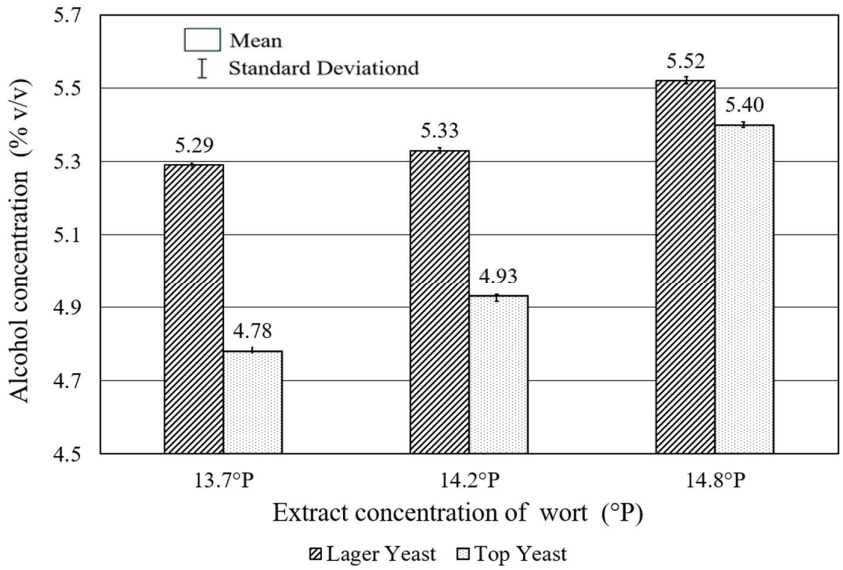

Figure 6. *Potential alcohol concentration of quinoa-based wort using the two yeast strains, LY and TY*

Therefore, based on the results, the use of the *TY* yeast strain could be recommended for the production of beers with a lower ethanol content.
Further compositional characterization of these quinoa-based worts represents a promising direction for future research, aimed at optimizing the technological potential of the three quinoa varieties for brewing application.

CONCLUSIONS

The experimental design proposed, in this study, for the basic technological evaluation of quinoa as a substitute for barley malt confirmed quinoa's versatility and its value as an alternative raw material in brewing. A substitution level of up to 40 % quinoa for barley malt is technologically feasible, even in the absence of added enzymes.
Despite comparable worts extract levels and viscosities the altered milling profiles of quinoa grist constituted the principal factor limiting lautering performance. The inadequate presence of husk material compromised mechanical stability of the filter bed, a deficiency not offset by residual malt barley at the tested inclusion rate.
These findings demonstrate that quinoa's structural properties at high replacement levels pose significant challenges to lautering, underscoring the need for process optimization - such as grist modification or filtration aids - to enable effective integration of quinoa in brewing formulations without compromising process efficiency.
From a fermentative perspective, the experimental results support the use of a bottom-fermenting yeast strain for quinoa-based worts. Among the three quinoa varieties tested and based on preliminary technological evaluations conducted at a high substitution level of 40 % *Pilsner* malt, white quinoa emerged as the most suitable alternative in terms of brewhouse yield.
However, this approach allows for the identification of future research directions aimed at harnessing the compositional benefits of the three distinct quinoa varieties in the development of novel and unconventional beer styles with unique functional, nutritional, and sensory attributes.

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