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The Impact of Enzyme Type, Temperature, and Grinding Roller Interval on Barley Malt-Based Beverages

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ABSTRACT

Background: Malt is a vital ingredient in the brewing and food industries, as it influences the sweetness, flavor, and color of the final products. The quality and soluble solids content of barley-derived malt depends on temperature, enzyme type, and grinding roller interval. This study aimed to determine the optimal conditions for maximizing the soluble solids in Iranian malt.

Methods: Malt samples were treated at 57 and 72 °C, employing glucan maltohydrolase (GMH) and glucohydrolase (GH) enzymes, and grinding roller intervals of 0.6, 0.8, and 1 mm. The malt samples were then subjected to chemical analyses, including Brix measurement, iodine test, and pH determination.

Results: The results showed that higher temperatures increased the Brix value and decreased the pH value of the malt samples. No significant differences in Brix and pH were observed between the GMH and GH enzymes. Smaller grinding roller intervals (0.6 mm) increased the Brix value and decreased the pH. The optimal conditions for maximizing the soluble solids content were 72 °C with the GMH enzyme and a 0.6 mm roller interval (Brix = 7.8 g/100 cc, pH = 5.32). The temperature significantly influenced the iodine test, confirming starch hydrolysis into sugar at 72 °C.

Conclusion: This study recommends reducing the grinding roller interval from 0.8 to 0.6 mm and increasing the temperature from 57 to 72 °C to significantly enhance the malting process. Therefore, using a 72 °C temperature and 0.6 mm roller gap is advised for better malting efficiency.

1. Introduction

Barley, scientifically as known "*Hordeum vulgare*," belongs to the *Gramineae* family, species *Sativum*. It is considered a strategic agricultural product and a major staple that can be grown worldwide under various climatic conditions, from regions near the North Pole to tropical areas near the equator (El-Hashash & El-Absy, 2019). Chemically, barley is rich in protein, carbohydrates, dietary fiber, minerals, and vitamins, making it a nutritious food that can be used to treat various ailments, including osteoporosis, gout, anemia, digestive problems, fever relief, cancer prevention, constipation relief, bloating reduction, and high cholesterol (Kaur et al., 2021; Zeng et al., 2024). Malting is the most important food application of barley due to the presence of a hull and specific chemical compounds that allow for desirable changes during germination. These characteristics make barley more suitable for malting than other grains (Mallett, 2014). Malt is a product derived from the germination of barley grains and the activity of natural enzymes present in the malted material. Malt finds applications in various food items such as dietary supplements, biscuits, crackers, breakfast cereals, baby food, pickles, and sauces, serving as a suitable substrate for the growth of fungi and yeasts (Kaur et al., 2021; Punia, 2020). Malting is a sophisticated biotechnological process involving a series of steps, including steeping, drying, germination, separating sprouts, and milling. This process involves controlled germination of cereals to induce specific

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physical and biochemical transformations within the grain, followed by stabilization through grain drying. These transformations occur through three crucial stages: steeping, which increases the grain's water absorption; germination, which supports embryo growth, enzyme synthesis, and partial endosperm breakdown; and kilning, which ensures the stability of the final product (Gupta et al., 2010; Narwal et al., 2020). The hot extraction process involves dissolving materials directly soluble in water and continuously hydrolyzing their enzymatic content, which is crucial for the type and quality of barley malt extract. Approximately 90-92% of the extract composition consists of soluble carbohydrates. If sufficient hydrolysis of the cell membrane and storage compounds of the endosperm's starch and protein does not occur, the extract yield percentage decreases due to inadequate gelatinization of starch granules and insufficient amylolytic enzymes (Jaeger et al., 2021; Jamar et al., 2011; Mahdavi et al., 2024). The crushing of malt significantly affects the yield of extraction and the amount of soluble carbohydrates. In this regard, hammer mills and roller mills with grooved rollers are widely used in the industry to reduce the size of malt particles. Roller mills, due to their lower energy consumption, uniform particle distribution, and quieter operation, are recognized as the best mills in this industry. On the other hand, Increasing the roller rotation speed and number of passes can enhance the reduced sugar content of the extract. Additionally, the damage to starch granules due to the reduced distance between rollers increases susceptibility to enzymatic attack, ultimately accelerating hydrolysis (Mousia et al., 2004; Yin Tan et al., 2023). During the malting process, the enzymatic activity of certain enzymes such as amylases, hydrolases, glucokinases, phosphatases, proteases, and phytases increases. These enzymes play a crucial role in the degradation of the cell wall of barley sprouts and increase the efficiency of malt production. Enzymes like GMH and GH, which belong to the hydrolase group of enzymes, facilitate the separation of D-glucopyranose from the non-reducing end of the starch. In other words, they induce the hydrolysis of amylopectin, resulting in the production of dextrins and an increase in the speed of malt extraction (Guerra et al., 2009). Considering that about 70% of the barley cell wall is composed of beta-glucan, GMH enzyme, breaking down the cell wall into soluble beta-dextrin, improves the endosperm of malt grains and accelerates filtration. It is worth mentioning that the most important factors affecting enzyme activity are temperature, pH, time, and extract concentration. In fact, in the hot extraction process, the temperature balance between the required temperature for the hydrolytic activity of the above-mentioned enzymes and the degree of their thermal inactivation is crucial (De Arcangelis et al., 2019; Guerra et al., 2009; Rittenauer et al., 2021). Additionally, the yield of malt extract depends on the characteristics of barley grains (protein content, starch positioning, and type of amylolytic enzymes). These factors significantly influence the extract yield and the amount of reducing sugars (Fox & Bettenhausen, 2023; Taheri-Kafrani et al., 2021). Langenaeken *et al.* (2019) investigated the gelatinization properties of small and large starch granules and discovered that small granules, which make up 8% of the total starch content, gelatinize at temperatures between 62 °C and 78 °C. This study emphasizes the importance of granule size during the malting process (Langenaeken et al., 2019). Previous researchers, such as Briggs *et al.* (1986), endeavored to improve the quality of malt extract through innovative methods. They conducted hot extraction, customizing thermal programming to suit the unique characteristics of the malt being studied. This involved a two-stage process: first, applying 50 °C for one hour, followed by 65 °C for an additional hour. Moreover, they innovatively utilized an extruder instead of a conventional mill for the extraction procedure (Briggs et al., 1986). Mousia *et al.* (2004) discovered that in the malting process, increasing the gap between mill rollers results in a reduced extraction of soluble solids from the malt (Mousia et al., 2004). This consequently prolongs the extraction time and ultimately leads to a lower product Brix. As evident from the complexity and involvement of numerous factors in this field, various studies mention different factors with different ranges, making it a fundamental challenge to identify the influential characteristics of barley and the malting process. On one hand, barley cultivars grown in Iran, such as *Hordeum vulgare*, exhibit reduced extraction of soluble solids and significantly increased separation time for spent grain compared to Dutch malt. This results in the formation of a cohesive, impermeable, and water-saturated mass, making separation a laborious task. Addressing this issue requires investigating various influential factors on malt production and minimizing the associated problems, considering the industry's needs and the lack of consensus in previous research. Unlike earlier studies that were typically narrow in focus on Iranian malt and lacked agreement, our research offers a comprehensive investigation of the collective impact of these factors on the yield of extracted solids from malt production. This innovative method not only improves our comprehension of Iranian malt production but also provides useful knowledge for industry processors, allowing them to boost efficiency and quality in contrast to conventional industrial malts on the market. Thus, the primary objective of this research has been to investigate the performance of hydrolase enzymes, particularly due to their high thermal stability, in breaking down and digesting cell walls, especially beta-glucans, in malt grains. Additionally, different roller mill gaps (intervals) were utilized for malt grain crushing, and various temperatures were employed for starch gelatinization during the malt extraction process. Subsequently, relevant tests (such as Brix, pH, and iodine tests) were conducted to demonstrate the effect and efficiency of each factor on the yield of soluble solids extracted from malt.

2. Materials and Methods

2.1 Materials

The raw materials used in this research were as follows: malt

from Shahd Zagros Co Shehrekord Iran; GH enzyme and GMH Enzyme from AEB Brewing Co, San Polo, Italy; and purified water obtained from the Behnoush Co, Tehran, Iran. The laboratory materials used in the research included filter paper and iodine solution from Merck, Darmstadt, Germany.

2.2 Statistical Population and Treatments

The population under investigation pertained to the level of soluble solid content obtained from malt at two temperatures, 57 °C and 72 °C, utilizing two enzymes, GH and GMH, and three different roller gaps, including 0.6, 0.8, and 1 mm. Table 1 indicates the treatments used in the research.

2.3 The process of producing solid materials extracted from malt

The process of producing solid materials extracted from malt commenced with the preparation and weighing of the required raw materials including malt, water, GH enzyme, and GMH enzyme (model AUY220, Shimadzu, Quioto, Japan). Then, according to the treatment table (Table 1), the necessary preparations were made. To prepare the solid soluble content extracted from malt, dry barley malt was passed through a grooved roller mill (model-MDDP, Bühler AG, Uzwil, Switzerland) with roller gaps selected of 0.6, 0.8, and 1 mm. The resulting materials from the mill were mixed with 6 L of water and transferred into an Erlenmeyer flask. Then, the contents of the Erlenmeyer flask were heated at different temperatures (57 °C for 40 min and 72 °C for 5 min), while GMH and GH enzymes were added at a rate of 0.05 g/kg of malt under these conditions. During this process (heat-time mechanism), the starch present in the malt was converted into sugars. Subsequently, the resulting materials, after filtration, were maintained at a temperature of 20°C (Iranmanesh & Salimi, 2018).

2.4 Chemical Tests for Malt

2.4.1 Measurement of Brix (Solid Soluble Extract)

To determine the Brix percentage of the solid soluble extract (from malt), a Brix refractometer (model PR-34A, Atago, Japan) was utilized. Firstly, the device was turned on, and its special cell was washed with distilled water. The instrument was then calibrated using water that was previously verified. After one more drying cycle, the sample was transported to the Brix refractometer cell and, the trigger button was activated to determine the Brix value of the malt in g/100 cc (Iranian National Institute of Standards and Industrial Research, 2007).

2.4.2 Measurement of pH

The pH of the malt samples was measured using a pH meter (model T123-9S Metrohm, Herisau, Switzerland) The device was activated, and its particular electrode was rinsed with distilled water. Then, it was calibrated with buffer solutions of pH 4 and 7. The pH meter electrode was rinsed again with distilled water and dried. Then, the electrode was placed into the sample, and the pH of the sample was determined after pressing the start button (Iranian National Institute of Standards and Industrial Research, 2007).

2.4.3 Iodine Test

The iodometric method was used to convert starch into sugar. In the process of malt extraction, as soon as the temperature reached 72°C, a drop of the extracted malt solution was added to a glass rod placed on a plate containing 0.02 M iodine. This test was performed at intervals of 5, 7, and 10 min. When the conversion of starch to sugar was complete, the yellow color of iodine remained (Iranian National Institute of Standards and Industrial Research, 2007).

2.5 Statistical analysis

The data obtained from the research was analyzed using a factorial experiment in the form of a completely randomized design. The means were compared using the Duncan multiple range test at a specified significance level of α=1% using SPSS version 27 software.

Table 1. Description of treatments. The treatments were divided into 12 different groups based on temperature, enzyme type, and milling roller interval

Treatment No.	Description
A1	Temperature: 57°C, enzyme: GMH, Milling roller interval: 0.6 mm
A2	Temperature: 57°C, enzyme: GMH, Milling roller interval: 0.8 mm
A3	Temperature: 57°C, enzyme: GMH, Milling roller interval: 1 mm
A4	Temperature: 57°C, enzyme: GH, Milling roller interval: 0.6 mm
A5	Temperature: 57°C, enzyme: GH, Milling roller interval: 0.8 mm
A6	Temperature: 57°C, enzyme: GH, Milling roller interval: 1 mm
A7	Temperature: 72°C, enzyme: GMH, Milling roller interval: 0.6 mm
A8	Temperature: 72°C, enzyme: GMH, Milling roller interval: 0.8 mm
A9	Temperature: 72°C, enzyme: GMH, Milling roller interval: 1 mm
A10	Temperature: 72°C, enzyme: GH, Milling roller interval: 0.6 mm
A11	Temperature: 72°C, enzyme: GH, Milling roller interval: 0.8 mm
A12	Temperature: 72°C, enzyme: GH, Milling roller interval: 1 mm

3. Results and Discussion

Results from Figure 1. a demonstrated that the Brix of the samples significantly increased with increasing temperature from 57°C to 72°C ($p < 0.01$). The lowest Brix value was 5.5 g/100 cc observed in the samples processed at 57°C and the highest value was 7.5 g/100 cc observed in the samples processed at 72°C. Investigations have shown that the effect of temperature on the activity of malt enzymes is very significant in the malt extraction process (Guerra et al., 2009; Hua & Yang, 2016).

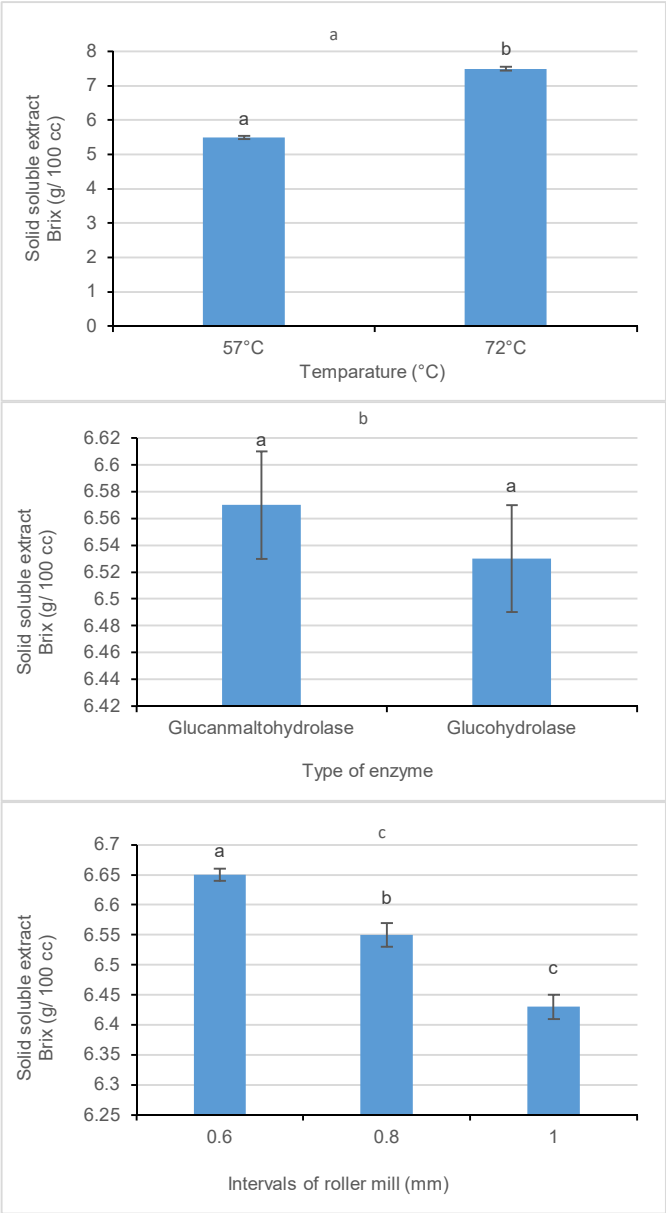

Figure 1. Evaluation of the results obtained from the effect of temperature, mill roller gap, and type of the enzyme on the Brix of samples. The effect of temperature (A), enzyme type (B), and mill roller gap (C). Different letters on each column indicate significant differences in the values of that column compared to others ($p < 0.01$)

In this research, the initial temperature of extraction to hydrolyze the beta-glucan wall in malt was considered to be 57 °C. Starch hydrolysis fails to produce sugar at this temperature due to incomplete hydrolysis. At temperatures of 72 °C, the gelatinization of starch and its hydrolysis occur completely, facilitated by GMH and GH enzymes. This process leads to a significant rise in Brix levels, despite their initial low concentration. The rise in temperature causes enzymes to attack starch granules, which increases the rate of starch hydrolysis, sugar production, and the Brix. The results of the research were consistent with the results of Brazil *et al.* (2019), who stated that the destruction of the beta-glucan wall by the GMH enzyme caused the complete hydrolysis of starch, which resulted in the formation of sugar and an increase in the Brix of the product (Brazil et al., 2019). Rimsten *et al.* (2002) also found that higher temperatures result in heightened malt enzyme activity, leading to the transformation of starch and insoluble compounds into water-soluble substances, thereby maximizing the production of simple carbohydrates. Furthermore, they noted that the effect of temperature is not only due to the increased enzyme activity but also due to the difference in the time required for soaking (Rimsten et al., 2002). Additionally, the increase in temperature plays a significant role in increasing Brix (Iranmanesh & Salimi, 2018). The results from Figure 1. b indicate that the addition of the GMH enzyme resulted in a higher Brix compared to the GH enzyme in the samples (GMH = 6.57, GH = 6.45), although this difference was not significant ($p > 0.01$). It appears that the use of hydrolase group enzymes, including GMH and GH, led to the separation of β-D-glucopyranose units from the non-reducing ends of the starch molecule. In other words, both enzymes caused the hydrolysis of amylopectin starch, resulting in the production of dextrin and increased malt extraction. Rani and Bhardwaj (2021), stated that hydrolase group enzymes, such as alpha-amylases and beta-amylases, play a vital role in breaking down and digesting the cell walls of malt grains due to their high thermal stability, leading to an increase in the amount of soluble solids. According to the results from Figure 1. c, the samples with the smallest roller gap (0.6 mm) achieved significantly higher Brix levels (6.65 g/100cc) compared to the other samples ($p < 0.01$). On the other hand, the samples with the largest roller gap of 1 mm had the lowest Brix levels. Specifically, the lowest Brix levels (6.43 g/100cc) were obtained in the samples processed with the largest roller gap. Additionally, the differences in Brix levels observed with varying roller gaps were significant ($p < 0.01$) (Rani & Bhardwaj, 2021). The results obtained in this study are consistent with those of other researchers regarding the impact of the distance (gap) between mill rollers on the final product's Brix. Studies have shown that during the grinding of malt grains, the greater the distance between the mill rollers, the lower the amount of soluble solids extracted from the malt, the longer the extraction time, and ultimately, the lower the product's Brix (Kharchenko et al., 2021; Mousia et al., 2004). The results of the interaction effect of temperature and enzyme on the Brix of samples are presented in Figure 2. a Based on these findings at temperatures of 57°C and 72°C, changing the type of enzyme (GMH or GH) did not lead to considerable differences in the Brix of the produced samples. The Brix values were 5.4 g/100cc for GH and 5.6 g/100cc for GMH at 57°C, 7.5 g/100cc for GH, and 7.6 g/100cc for GMH at 72°C. Although there was a slight quantitative increase in the Brix of the product in the presence of GMH enzyme compared to GH enzyme, this difference was not statistically significant ($p > 0.01$). In other words at constant temperatures changing the type of enzyme had little effect on the Brix value. Moreover, the temperature rise had a substantial impact on the Brix levels of the samples when either enzyme was

employed ($p < 0.01$). Overall, it can be stated that increasing the temperature has a higher impact on the Brix of the product compared to changing the type of enzyme (Iranmanesh & Salimi, 2018; Toffoli et al., 2003). The results of the interaction effect of temperature and the distance between rollers on the Brix of samples are presented in Figure 2. b It can be concluded that at temperatures of 57°C and 72°C, increasing the distance between the mill rollers significantly led to a decrease in the Brix of the produced samples. This significant difference was observed between the treatments with the smallest roller gap (0.6 mm) and the treatments with the largest roller gap (1 mm) ($p < 0.01$). Specifically, at 57°C, the treatment with the smallest roller gap (0.6 mm) had the highest Brix value (5.6 g/100cc), while the treatment with the largest roller gap (1 mm) had the lowest Brix value (5.4 g/100cc). Similarly, at 72°C, the treatment with the smallest roller gap (0.6 mm) had the highest Brix value (7.7 g/100cc), and the treatment with the largest roller gap (1 mm) had the lowest Brix value (7.45 g/100cc). Notably, the difference between these two treatments was significant ($p < 0.01$). Additionally, the group with a roller gap of 0.8 mm showed Brix values of 5.5 and 7.6 g/100cc at 57°C and 72°C, respectively. These values were lower than those of the group with a roller gap of 0.6 mm but higher than those of the group with a roller gap of 1 mm. These differences were statistically significant ($p < 0.01$). Moreover, at identical roller gaps, increasing the temperature from 57°C to 72°C significantly increased the Brix of the samples. Specifically, the treatment with a temperature of 72°C and a 0.6 mm roller gap had the highest Brix, while the treatment with a temperature of 57°C and a 1 mm roller gap had the lowest Brix overall ($p < 0.01$). Eneje *et al.* (2001) stated that the roller gaps affect the quality of extraction from malt, such that as the distance between the mill rollers increases, the amount of soluble solids extracted from the malt decreases. They also noted that changing the roller gap affects the extraction rate and the amount of soluble carbohydrates. In other words, they indicated that the distance between the rollers, by influencing the production of finer or coarser particles, can impact the amount of extract obtained from malt (Eneje et al., 2001). These findings are consistent with the results of the present study.

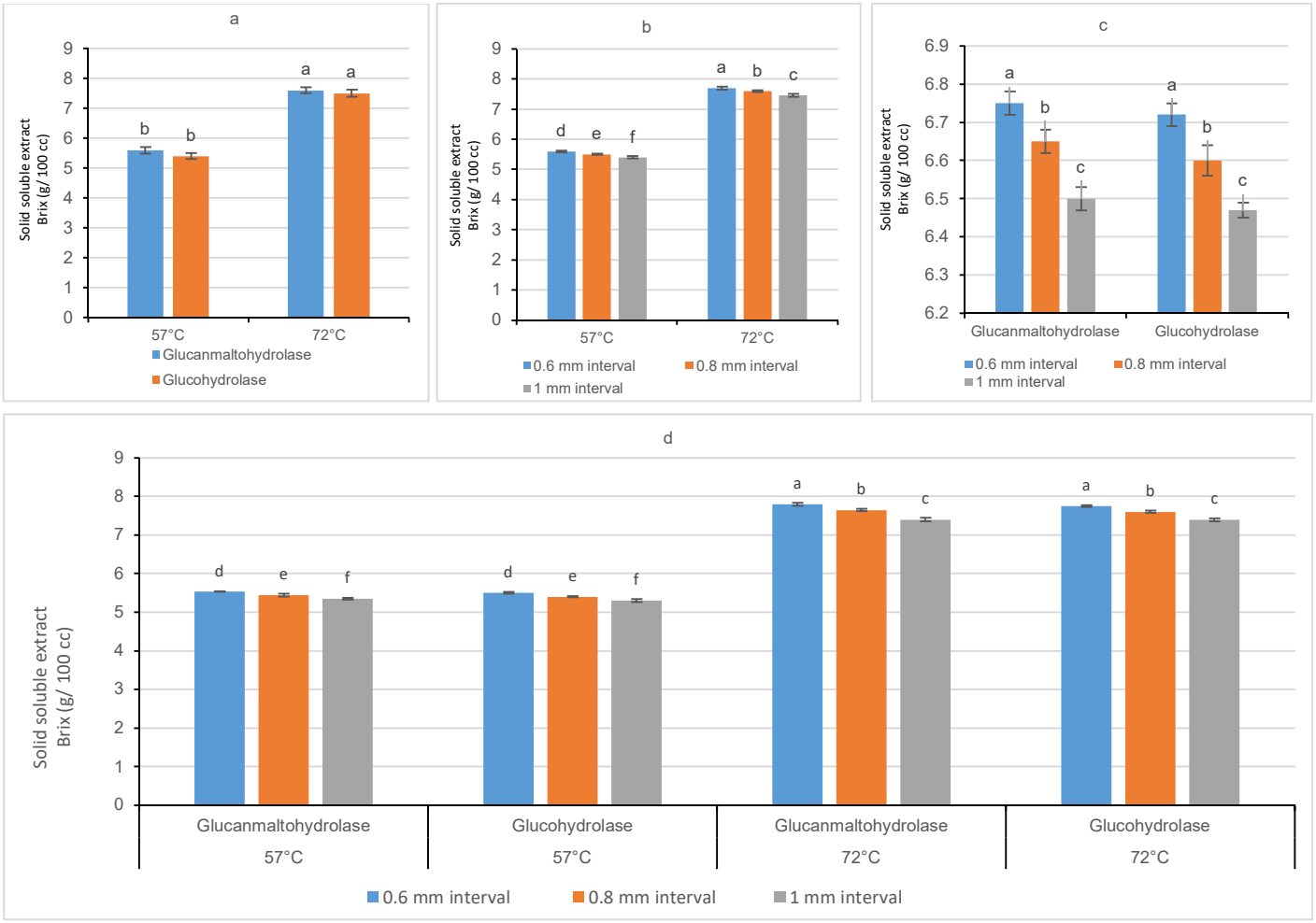

Figure 2. Evaluation of the results obtained from the interaction effect of temperature, mill roller gap, and type of the enzyme on the Brix of samples. The interaction effect of temperature and enzyme type (a), temperature and mill roller gap (b), enzyme type and mill roller gap (c), temperature, enzyme type, and mill roller gap (d). Different letters on each column indicate significant differences in the values of that column compared to others ($p < 0.01$)

The results obtained from the interaction influence of enzyme and roller gap distance on the Brix of samples in Figure 2. c indicate that in samples containing each type of enzyme (GMH and GH), an increase in roller gap distance leads to a decrease in the Brix of samples by 0.25 g/100cc ($p < 0.01$). On the other hand, with consistent roller gap distances, changing the type of enzyme did not result in a statistically significant difference in the Brix values of samples ($p > 0.01$). In terms of enzyme performance, although no significant difference was observed in the Brix created in samples by GMH compared to those prepared with GH enzyme, the Brix was slightly higher in samples prepared with GMH. In general, it can be concluded that the roller gap distance in the Brix of samples has more significance compared to the type of enzyme used Kihara *et al.* (2002), stated that enzyme types such as GH and GMH affect the efficiency of malt production in terms of the amount and type of maltose (Kihara et al., 2002). The results obtained from the mutual influence of temperature, enzyme, and roller gap distance (interval) on the Brix of samples in Figure 2. d indicate that at temperatures of 57°C and 72°C, and with the addition of each of the researched enzymes (GMH and GH), an increase in roller gap distance has led to a decrease in the Brix of samples ($p < 0.01$). Generally, the highest Brix values were observed in samples processed with a roller gap distance of 0.6 mm accompanied by the GMH enzyme at 72 °C (7.8 g/100cc), while the lowest Brix values were observed in samples processed with a roller gap distance of 1 millimeter accompanied by the GH enzyme at 57 °C (5.3 g/100cc). This aligns with findings from other studies highlighting the significant role of roller gap distance, particularly in conjunction with temperature, on the Brix index. This influence was also evident in our study (Iranmanesh & Salimi, 2018; Rani & Bhardwaj, 2021; Rimsten et al., 2002). The results obtained from the effect of temperature, distance between the mill rollers, and enzyme on the pH of the samples in Figure 3. a indicate that with an increase in temperature from 57°C to 72°C, the pH of the samples decreased from 6.18 to 5.79 ($p < 0.01$). The current investigation selected a temperature of 57 °C as the beginning extraction temperature for the hydrolysis of the malt's cell wall. At this temperature, the GMH enzyme was unable to completely hydrolyze the starch and release sugar, resulting in partial starch hydrolysis. Therefore, the brix decreased, and due to the lack of the Maillard reaction, its pH did not decrease significantly. However, as soon as the temperature increased to 72°C, the gelatinization and hydrolysis of starch intensified. Moreover, with the increase in temperature and hydrolysis, the conditions for the initiation of the Maillard reaction were provided, which led to a decrease in pH. Since the Maillard reaction occurs at an alkaline pH and the amino group is consumed in this process, the pH of the product decreases (echovská et al., 2012; Palmer, 2017). Coghe *et al.* in 2006 stated that during the drying stage, considering the temperature used, the intensity of the Maillard reaction would vary, and in line with this, the use of higher drying temperatures led to a decrease in the pH of the product (Coghe et al., 2006). As illustrated in Figure 3. b, the effect of enzymes on the pH of the samples was investigated. The results showed that the use of GH and GMH enzymes did not cause a significant difference in the pH of the samples ($p > 0.01$). One possible reason for this is that there was no significant change in the Maillard reaction under these conditions, and therefore the pH of the product did not change significantly (Van Boekel, 2001). Figure 3. c, notes the effect of the roller mill gap on the pH of the samples was investigated. The results showed that the sample with the smallest gap (0.6 mm) had a significantly lower pH than the samples with larger gaps (0.8 and 1 mm) ($p < 0.01$). There was also a significant statistical difference between the samples with gaps of 0.8 and 1 mm ($p < 0.01$). In total, a maximum decrease of 0.48 in pH was observed with decreasing roller gap. Natoniewski *et al.* (2018), stated that the smaller the gap between the roller mills, the higher the amount of soluble solids extracted from the malt and the greater the enzyme attack due to damage to the starch granules, resulting in a higher rate of starch hydrolysis. This leads to more reactions and ultimately a decrease in pH (Natoniewski et al., 2018).

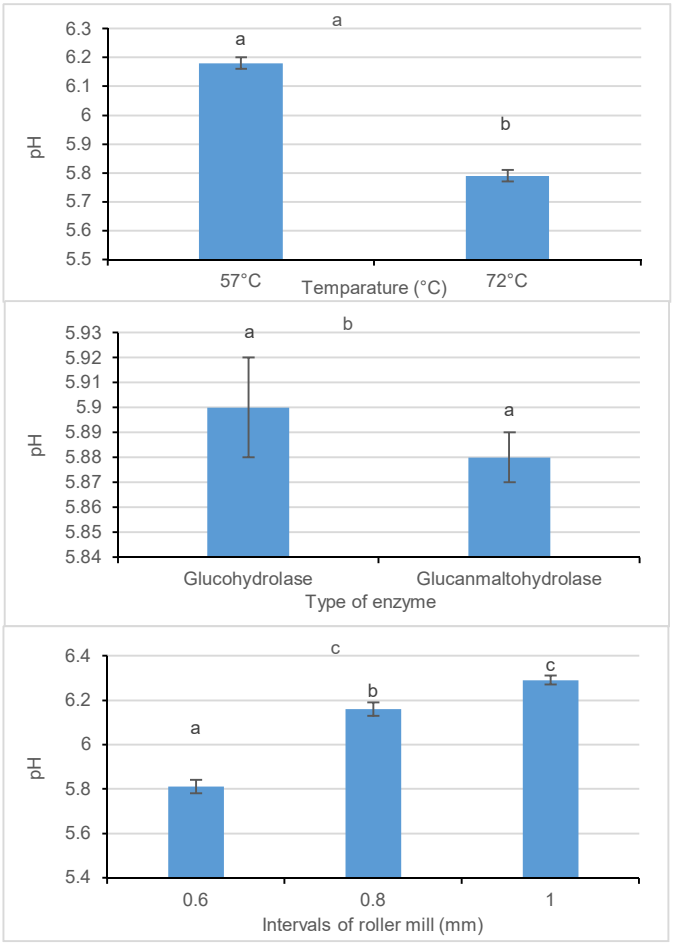

Figure 3. Evaluation of the results obtained from the effect of temperature, mill roller gap, and type of enzyme on the pH of samples. The effect of temperature (a), enzyme type (b), and mill roller gap (c). Different letters on each column indicate significant differences in the values of that column compared to others ($p < 0.01$)

Figure 4. a, illustrates the interaction between temperature and enzyme on the pH of the samples. The highest pH value of 6.09 was observed in the treatment process with GH enzyme at 57 °C. The lowest pH value of 5.70 was observed in the group containing the GMH enzyme. However, there was no significant statistical difference in the pH factor of the produced samples at 57 and 72 °C with the change of enzyme type (GMH and GH) ($p > 0.01$). In addition, in the samples processed with the same enzyme, an increase in temperature led to a decrease in pH, which was statistically significant ($p < 0.01$). Previous researchers have stated that increasing the temperature leads to an increase in the attack of enzymes on starch granules, resulting in a high rate of starch hydrolysis and sugar formation. Due to the Maillard reaction and the consumption of amino groups of proteins, the pH decreases (Coghe et al., 2006; Ekielski et al., 2018). As displayed in Figure 4. b, the interaction between temperature and roller mill gap on the pH of the samples was investigated. The results showed that at temperatures of 57 and 72 °C, increasing the roller mill gap led to an increase in pH ($p < 0.01$). However, at 72 °C, there was a significant statistical difference in the pH of samples with different roller mill gaps ($p < 0.01$). The lowest pH (pH = 5.39) was observed in samples with a 0.6 mm roller mill gap at 72 °C, while the highest pH (pH = 6.31) was observed at 57 °C with a 1 mm roller mill gap. On the other hand, at the same roller mill gaps, the pH of the samples decreased significantly with increasing temperature ($p < 0.01$).

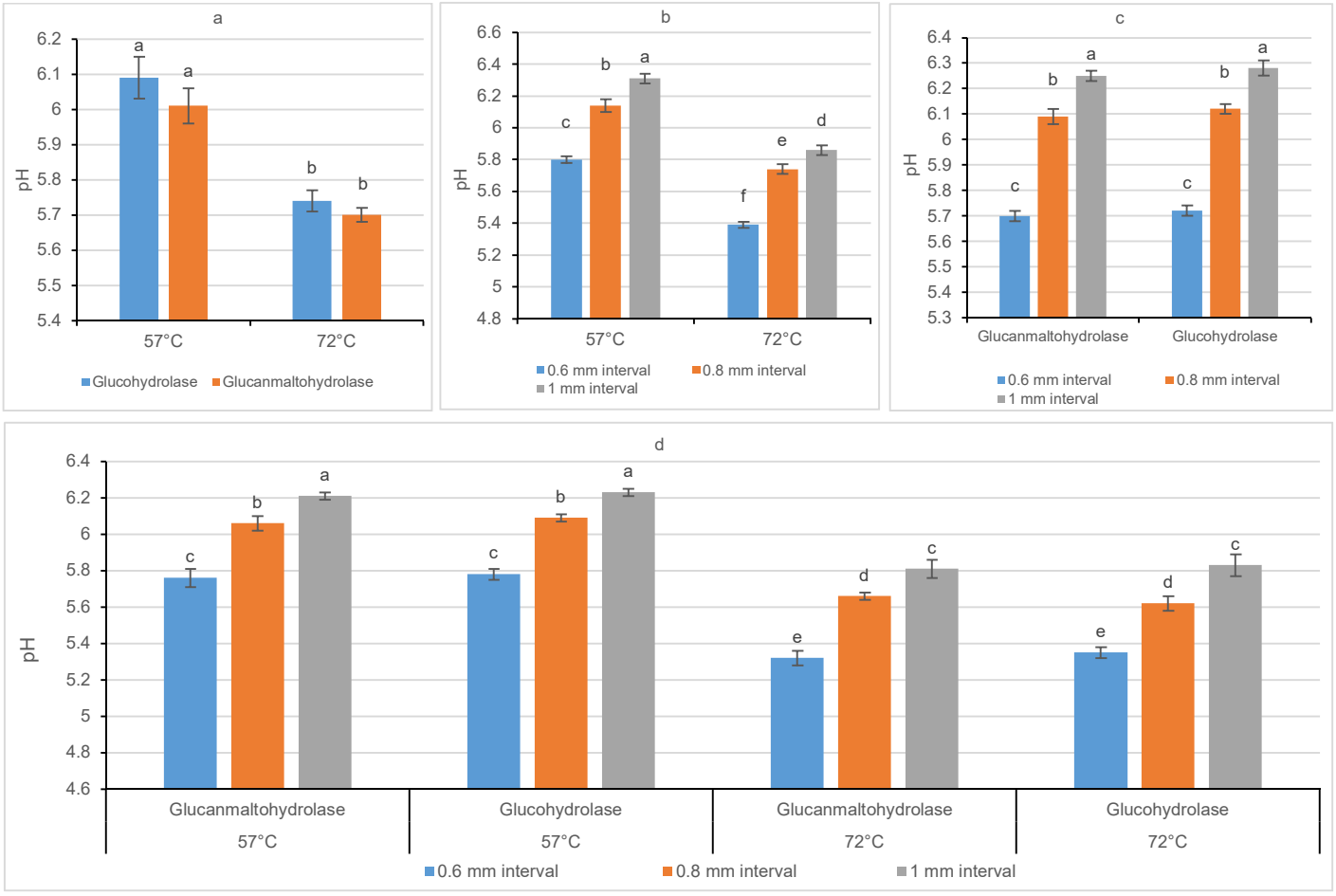

Figure 4. Evaluation of the results obtained from the interaction effect of temperature, mill roller gap, and type of enzyme on the pH of samples. The interaction effect of temperature and enzyme type (d), temperature and mill roller gap (e), enzyme type and mill roller gap (f), temperature, enzyme type, and mill roller gap (g), on the pH of samples. Different letters on each column indicate significant differences in the values of that column compared to others ($p < 0.01$)

The results of this study are consistent with those of other similar studies in this field, which have shown that decreasing particle size or increasing temperature increases the reaction of compounds and their surface area for further reaction (more Maillard reaction), which leads to a decrease in pH, which is consistent with the results of this study (Coghe et al., 2006; Ekielski et al., 2018; Natoniewski et al., 2018). As presented in Figure 4. c, the interaction between the enzyme and roller mill gap on the pH of the samples was investigated. The results showed that when using either GMH or GH enzymes, increasing the roller mill gap led to an increase in pH. This difference was significant when comparing different roller mill gaps using GMH and GH enzymes ($p < 0.01$). With a change in roller gap from smallest

to largest for GMH and GH enzymes, pH increased by 0.55 and 0.56, respectively. On the other hand, at fixed roller gaps, there was no significant decrease in pH when the enzyme type was changed from GH to GMH ($p > 0.01$). As shown in Figure 4. d, the interaction between temperature, enzyme, and roller mill gap on the pH of the samples was investigated. The results showed that at 57 °C, the pH of the samples increased with increasing roller mill gap using both types of enzymes (GMH or GH) ($p < 0.01$). The highest pH values for the samples at this temperature were recorded at a roller mill gap of 1 mm, with values of 6.23 and 6.21 when using GH and GMH enzymes, respectively. Also, at 72 °C, the use of a smaller roller mill gap in the treatments using GH and GMH enzymes resulted in a statistically significant difference ($p < 0.01$). The pH of the samples decreased significantly with decreasing roller mill gap and increasing temperature. The maximum decrease in pH for the samples due to these treatments was 0.89, which is a significant amount. Consistent with these findings, Schwarz *et al.* (2007) investigated the impact of operational parameters on the determination of laboratory extract and yeast quality factors. They concluded that milling had the most significant effect among the parameters, influencing other analytical values. Temperature also impacted all analytical parameters, with a more pronounced effect on yeast color, β-glucans and fermentable sugars (Schwarz et al., 2007). Several studies have also shown that at small roller mill gaps, starch granules are more damaged and therefore more susceptible to enzyme attack, resulting in increased starch hydrolysis and sugar formation. With the destruction of the beta-glucan wall by the GH enzyme, complete hydrolysis of starch and sugar formation by hydrolases leads to increased Brix, the iodine yellow color remains constant (negative iodine test), and pH decreases, which is in high agreement with the findings of this study (Natoniewski et al., 2018; Rittenauer et al., 2021; Toffoli et al., 2003; Yin Tan et al., 2023). Therefore, in this study, it can be stated that the interaction of enzyme use, increased temperature, and decreased roller mill gap led to increased starch hydrolysis and reduced sugar formation, which ultimately accelerated the Maillard reaction and decreased pH. According to the results of the interaction between temperature, enzyme, and roller mill gap on the iodine test of the samples in Table 2, at 57 °C, with different roller mill gaps (0.6, 0.8, and 1 mm) and/or with different enzyme types (GH and GMH), the iodine test was positive for all samples. At this temperature, the enzymes are unable to break down the starch into sugar entirely, and the GMH enzyme can't cause much damage to the grains, so it is prohibited from attacking them. As a result, the starch is not fully hydrolyzed, and the iodine color remains yellow-black, resulting in a positive iodine test. However, the iodine test was negative for all samples at 72 °C with different roller mill gaps (0.6, 0.8, and 1 mm) and/or different enzyme types (GH and GMH). Generally, the iodine test (iodometric technique) is used to assess the conversion of starch to sugar. Once the conversion of starch to sugar is complete, the iodine color remains yellow (Fleischer, 2019; Sebestyén et al., 2013). A negative iodine test implies the completion of starch breakdown by enzymes. Hot extraction aims to prolong the effectiveness of malt enzymes and transform starch and insoluble substances into water-soluble compounds to maximize the production of simple carbohydrates. Consequently, a negative iodine test is deemed favorable (Contreras Jiménez et al., 2019; Karki & Kharel, 2012) which was achieved at a temperature of 72 in our research.

Table 2. Interaction effect of temperature, enzyme, and Intervals of roller mill on iodine test of samples

Temperature (°C)	Type of enzyme	Intervals of roller mill (mm)		
		0.6	0.8	1
57°C	Glucanmaltohydrolase	+	+	+
	Glucohydrolase	+	+	+
72°C	Glucanmaltohydrolase	-	-	-
	Glucohydrolase	-	-	-

+: Positive -: Negative

4. Conclusion

Barley malt, obtained from the sprouting of barley, has wide applications in various industries such as confectionery, caramel, and sweet production, as well as the production of vinegar, alcohol, breakfast cereals, and various beverages such as soft drinks and malt-based beverages. The solid content and quality of the extracted malt are influenced by various factors such as temperature, enzyme type, and the gap between the mill rollers. This study investigated the effects of these factors on the Brix and pH of the produced malt compared to ordinary industrial malts. Regarding the Brix factor, increasing the temperature to 72°C and decreasing the gap between the rollers to 0.6 mm had the most significant effect. The highest Brix values were obtained in the samples processed with a roller gap of 0.6 mm and at a temperature of 72°C using the enzymes GMH and GH. Concerning the pH factor, increasing the temperature from 57°C to 72°C decreased the pH of the samples. Also, the sample with the smallest gap between the mill rollers (0.6 mm) had a significantly lower pH than the samples with a larger gap between the mill rollers (0.8 and 1 mm). Moreover, at a constant temperature and gap between the mill rollers, changing the enzyme type did not result in any statistically significant difference in the pH and Brix values of the produced samples. The iodine test was also negative at a temperature of 72°C, regardless of the enzyme type and the gap between the rollers. At 57°C, even with a smaller gap between the rollers, the conditions for enzyme action and conversion of starch to sugar were not met. It should be noted that due to the limited time and laboratory resources, increasing the range of parameters would significantly increase the number of treatments, making it practically impossible to perform a larger number of experiments. Therefore, it is suggested that the effect of other temperatures on the pH and Brix factors of milled malt be investigated. Moreover, investigating other gaps between the mill rollers and their effect on the pH and Brix factors of the malt can lead to valuable information. Furthermore, the effect of the beta-amylase enzyme on the Brix factor of milled malt can be investigated in future research.

Authors' Contributions

Mohammadreza Iranmanesh: Investigation; Writing-original draft. Abolfazl Kamkar: Writing-review & editing; supervision. Ali Misaghi: Writing-review & editing; validation. Hassan Gandomi: data curation; validation. Solmaz Saremnezhad: Investigation; data curation. Ramin Khorrami: conceptualization; Investigation; Writing -original draft.

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Conflicts of Interest

The authors declare no conflict of interest.

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Ethical considerations

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