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Harnessing Triticale (× *Triticosecale* Wittmack) Malting for Amylase Enzyme Synthesis and Partial Purification

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ABSTRACT

Triticale malt is an underutilized cereal matrix with high amylolytic potential, which may be exploited for the recovery of plant-derived enzymes for food and biotechnological applications. This study aimed to obtain α-amylase- and β-amylase-enriched aqueous extracts from Bicentenario and Siglo XXI triticale malts and to partially purify these enzymes using ethanol/trisodium citrate aqueous two-phase systems (ATPS). Triticale grains were malted by germination for 5 days and dried at 50°C. Aqueous extraction was performed using ground malt and deionized water at a 1:10 ratio under different extraction times (30, 120, and 270 min) and temperatures (30°C and 40°C). The selected extracts were lyophilized and subjected to ATPS composed of ethanol and trisodium citrate. Enzyme activity was determined using the BETAMYL-3 method for β-amylase and the AOAC 2002.01, AACC 22-02.01, and ICC Standard No. 303 methods for α-amylase. Mild extraction conditions favored enzyme recovery, with the highest α-amylase activity observed in Siglo XXI extract at 30 min/30°C, increasing from 99.10 CU/g in malt to 507.42 CU/g in the aqueous extract. β-Amylase activity was also higher in Siglo XXI than in Bicentenario extracts. The 30% ethanol/18% trisodium citrate ATPS was the most effective system, promoting differential partitioning with α-amylase concentrated in the bottom phase (432.89 CU/mL) and β-amylase in the top phase (5.78 BU/mL). These results highlight the potential of triticale malt as a renewable source of amylolytic enzymes and support ethanol/trisodium citrate ATPS as a mild, sustainable strategy for obtaining amylase-enriched fractions, although further optimization is needed to improve β-amylase recovery and scalability.

1 | Introduction

Triticale (× *Triticosecale* Wittmack) is an anthropogenic cereal that combines the high yield and functionality of wheat (*Triticum* spp. Linnaeus 1753) with the resilience of rye (*Secale cereale* Linnaeus 1753). This grain stands out for its high amylolytic activity compared to other cereals and is considered a food with potential for human consumption due to its high nutritional value, which is more similar to that of wheat, although its applications are currently limited to animal feed and minor brewing uses [1, 2]. Because of this high amylolytic activity, triticale has been used to increase the yield of barley malt worts, although viscosity and fermentable extract are affected when at least 10% of the malted barley is replaced with triticale [3, 4]. In addition to these advantages, triticale is a cost-effective crop to produce, which enhances its viability as a raw material in industrial applications.

Malted cereals serve as a significant biological platform for enzyme production, as germination activates the endogenous

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hydrolytic enzymes involved in starch mobilization. In this context, malted triticale is an attractive yet underutilized source of amylolytic enzymes. Although recent studies have evaluated malted triticale as a functional ingredient in cereal-based foods, its use as a direct source of recoverable α-amylase and β-amylase remains limited [5]. Nevertheless, its ability to produce large amounts of α-amylase and β-amylase makes triticale a viable raw material for the production of enzymatic extracts with possible industrial applications [6]. In the brewing and starch industry, α-amylase and β-amylase preparations are widely used to enhance fermentable sugar production, improve wort quality, and support the development of value-added products. In this context, malted triticale may represent an alternative plant-derived source of amylolytic enzymes for food and beverage applications [7]. Although optimized microbial systems generally achieve higher volumetric activities [8, 9], plant-based platforms remain relevant due to their renewable origin, mild processing requirements, and potential food-oriented applications, as reported for α-amylase from germinated *Cucurbita moschata* seeds [10].

Plant-derived enzymes have gained attention as sustainable and biocompatible alternatives for food and industrial bioprocesses. However, their application is still limited by factors such as low recovery, instability during extraction, and the presence of interfering compounds, making it necessary to identify plant matrices with high enzymatic potential and efficient recovery strategies [11]. Consequently, triticale malt has the potential to contribute to this field not only as a brewing ingredient but also as a plant-based source of amylolytic enzymes.

The recovery of amylolytic enzymes with high activity from cereal kernels has been shown to be economically viable using medium-resolution purification techniques, such as aqueous two-phase systems (ATPS) [12]. Similarly, Leykun et al. [13] reported the recovery of over 98% of thermostable lipase activity using an alcohol–salt ATPS, underscoring the efficacy of these systems in simplifying downstream enzyme processing. These systems are efficient for extracting and recovering biomolecules from aqueous extracts and biological materials and are more cost-effective than other purification techniques. In this regard, a recent techno-economic meta-analysis demonstrated that phase separation-based processes, including ATPS, can be more cost-effective than chromatographic operations, particularly at moderate-to-large production scales and when processing low-purity feedstocks, where chromatography becomes economically restrictive [14]. In addition, ATPS are considered green and mild liquid–liquid extraction platforms because they commonly form water-rich phases, reduce the need for toxic organic solvents, and help preserve biomolecule activity during recovery [15]. Their simplicity, cost-effectiveness, scalability, and biocompatibility support their use for the isolation, purification, and enrichment of biomolecules such as proteins and enzymes [16]. Moreover, their adaptability to continuous operation further supports their potential as an environmentally friendly alternative for producing purified products at industrial scale [17, 18].

Notable studies in this field include the recovery of glutenins from wheat seeds, recombinant proteins from alfalfa [19], and industrial enzymes such as α-amylase and β-amylase from malted maize extracts, achieving high activity recovery in PEG/$CaCl_2$ systems. The importance of these studies lies in the added value they can bring to cereals such as triticale or maize, which can generate significant amylolytic activity for industrial applications [12, 20]. However, available information on the integrated use of triticale malting, aqueous extraction, and ethanol/trisodium citrate ATPS for the recovery of α-amylase- and β-amylase-enriched fractions remains limited.

Therefore, the novelty of this study lies in evaluating two triticale varieties, Bicentenario and Siglo XXI, as enzymatic raw materials and coupling mild aqueous extraction with ethanol/trisodium citrate ATPS as a sustainable downstream strategy. For the aforementioned reasons, this study aims to obtain aqueous extracts from Bicentenario and Siglo XXI triticale malts enriched in β-amylase and α-amylase, and to partially purify them using ATPS, proposing triticale as a viable, sustainable, and underutilized plant-based source of amylases for the food industry.

2 | Materials and Methods

2.1 | Biological Material

Seeds of triticale (× *Triticosecale* Wittmack) of the varieties the Bicentenario (BTO) and Siglo XXI (SXXI), both with germination rates > 95%, were used. The seeds were harvested in 2017 from experimental fields of the Instituto de Investigación y Capacitación Agropecuaria, Acuícola y Forestal del Estado de México (ICAMEX), located in Metepec, Estado de México, Mexico. These varieties were selected because ICAMEX recommends Siglo-XXI and Bicentenario for forage and grain production in the State of Mexico, highlighting their agronomic relevance, disease resistance, and, in the case of Bicentenario, grain yields of 4.0–6.0 t/ha under suitable conditions [21].

2.2 | Malt Production

To obtain diastatic malt with high α-amylase and β-amylase activity, 100 g of seeds (BTO or SXXI) was washed using a pH 7.0 non-ionic detergent and subsequently disinfected with a 1% $Ca(ClO)_2$ solution. The seeds were then immersed in deionized water for 2-h intervals, with a 30-min break between each interval, until reaching a moisture content of 45% ± 1%. The seeds were then germinated for 5 days at 17°C and 100% relative humidity [22] in a germination chamber (Seedburo model SEARS 255.94299110). The germinated seeds were then dried at 50°C in a hot air circulating oven (Riosa model HCF-62D) until a moisture content of approximately 7% was reached [23]. The seeds were ground in a rotor mill (Retsch GmbH ZM 300), sieved (mesh No. 60) [24], and stored for further analysis in plastic bags.

2.3 | Malt Characterization

Moisture and protein content analyses were performed using the Malt-3 and Malt-8 methods [24]. Reducing sugars were quantified using the DNS method, with absorbance measured at 540 nm using a UV–Vis spectrophotometer [25], total solids were determined by gravimetric analysis [26], protein content was assessed using the bicinchoninic acid (BCA) assay (Thermo Fisher Scientific), with absorbance measured at 562 nm, and amylase activity was measured using Megazyme kits: α-amylase (AOAC 2002.01, AACC 22-02.01, and ICC Standard No. 303 methods) and β-amylase (BETAMYL-3 method).

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TABLE 1 | Average values for moisture, reducing sugars, protein, β-amylase, and α-amylase in BTO and SXXI triticale malts.

Malt type	Moisture (%)	Reducing sugars (mg/mL)	Protein (%)	β-Amylase (BU/g)[b]	α-Amylase (CU/g)[c]
Bicentenario	6.64 ± 0.16^{a}	8.94 ± 0.04^{a}	11.38 ± 0.09^{a}	28.63 ± 0.01^{b}	112.9 ± 0.49^{a}
SXXI	6.50 ± 0.17^{a}	7.67 ± 0.04^{b}	12.38 ± 0.18^{b}	37.16 ± 0.02^{a}	99.10 ± 1.08^{b}

[a]Values are expressed as mean ± standard deviation ($n = 3$). For each variable, means followed by different superscript letters within the same column differ significantly according to Student's t-test for independent samples ($p \leq 0.05$).
[b]BU/g: It is defined as the amount of enzyme, in the presence of an excess of thermostable β-glucosidase, required to convert 1 μmol of p-nitrophenol from PNPβ-G3 in 1 min under defined assay conditions.
[c]CU/g: It is defined as the amount of enzyme, in the presence of an excess of thermostable α-glucosidase, required to convert 1 μmol of p-nitrophenol from BPNPG7 in 1 min under defined assay conditions.

2.4 | Aqueous Extraction of Amylases

Aqueous extracts were prepared by mixing ground malt (BTO or SXXI) with distilled water at room temperature (≈20°C) at a 1:10 (w/v) ratio in 20-mL polypropylene tubes. The mixture was homogenized using a vortex mixer (Labnet VX-200) at maximum speed for 10 min [27], followed by incubation in a floor incubator with orbital shaking (LUZEREN PRO1001322) at 180 rpm. The extraction conditions (treatments) included temperatures of 30°C and 40°C, combined with incubation times of 30, 120, and 270 min (30°C/30 min (T1), 30°C/120 min (T2), 30°C/270 min (T3), 40°C/30 min (T4), 40°C/120 min (T5), and 40°C/270 min (T6)). Subsequently, the tubes were centrifuged (10, 000 × g, 4°C, 10 min) and the supernatant was vacuum-filtered through Whatman No. 41 filter paper. The extracts were then frozen at −80°C for 36 h using an ultra-freezer (Thermo Scientific TSX40086A) and lyophilized for 48 h using a freeze-dryer (Huanghua Faithful FSF-18N) at −50°C under a vacuum pressure of 0.1 mbar to obtain solid samples. These samples were then subjected to α-amylase and β-amylase activity assays, as described in Section 2.3 "Malt Characterization."

2.5 | Amylase Purification

The aqueous extracts from each triticale variety (BTO and SXXI) with the highest amylolytic activity were selected for enzymatic liquid–liquid partitioning using 5 g ATPS formulated with ethanol (≥ 99.8%, Merck, Germany) and trisodium citrate (SC) (Merck, Germany) at concentrations of 45%/10%, 35%/13%, and 30%/18%, according to the binodal curve reported by Ooi et al. [28]. Each ATPS was prepared with 10% (0.5 g) lyophilized extract and deionized water. The α-amylase and β-amylase activities were assayed in each phase of the ATPS as described in Section 2.3 "Malt Characterization." Partition parameters, including the protein partition coefficient (K_p), enzymatic activity partition coefficient (K_e), selectivity (S), purification factor (F_p), and theoretical yield (Y), were calculated to evaluate the performance of the ATPS [29].

2.6 | Experimental Design and Statistical Analysis

For all data, parametric tests for normality, independence, and homoskedasticity were conducted. The experimental design for malts was obtained through a completely randomized design with variety as the independent factor and moisture (%), total solids (%), reducing sugars (mg/L), protein (%), and α-amylase and β-amylase activities as response variables. The data were subjected to analysis using Student's t-test. The aqueous malt extracts design was obtained using a randomized block factorial design (2∗3) with temperature (30°C and 40°C) and extraction time (30, 120, and 270 min) as independent variables, and the same response variables as for malts. Data were analyzed using a factorial ANOVA ($p \leq 0.05$). In addition, a multivariate analysis was performed to explore correlations between the factors and response variables. For the ATPS, a completely randomized design was used with ATPS composition as the independent with 3 levels for ethanol and SC (45%/10%, 35%/13%, and 30%/18%), where α-amylase and β-amylase activities were the response variables. The obtained data were analyzed by ANOVA ($p \leq 0.05$). When significant differences ($p \leq 0.05$) were identified, Tukey's multiple comparison test was used. All treatments were performed in triplicate. Statistical analysis was carried out using Statgraphics Centurion XV software.

3 | Results and Discussion

3.1 | Malt Analysis

The moisture content of diastatic malts from the BTO (6.64 ± 0.16%) and SXXI (6.50 ± 0.17%) varieties did not show statistically significant differences ($p \leq 0.05$) (Table 1) and remained within the optimal range for diastatic malts, typically between 6% and 7%. This range is achieved by drying at temperatures below 100°C, which helps maintain diastatic power (enzymatic activity) and preserve the structural integrity of residual polysaccharides [30].

Significant differences were observed ($p \leq 0.05$) for reducing sugars content, with values of 8.94 mg/mL for the BTO and 7.67 mg/mL for SXXI (Table 1). These concentrations were significantly lower compared to those reported for malts derived from barley, wheat, or rye (16.5–20 g/kg) [31]. The relationship between reducing sugars and enzymatic activity in malts is positively correlated and varies across cereal species [7, 32]. Furthermore, the type of cereal and the malting protocol exert significant influence on the activation of amylolytic enzymes during germination, resulting in sequential hydrolysis of starch and variable reducing sugar concentrations [30, 32].

Regarding protein content, the BTO variety (11.38%) had a significantly higher protein concentration ($p \leq 0.05$) than the SXXI malt (12.38%), with both values falling within the optimum range for diastatic malts (10.8%–12.3%). It is well documented that protein content in cereals is closely associated with enzyme synthesis during germination, particularly with respect to free amino nitrogen (FAN) levels, as well as other factors such as the aleurone layer and gibberellic acid [32]. A low total protein concentration would be associated with a lower amount of amylolytic enzymes. Previous studies have shown that triticale

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TABLE 2 | Mean values for total solids, reducing sugars, and protein in the aqueous extracts of triticale malt from the SXXI and BTO varieties[a].

Triticale malt variety	Treatment	Extract total solids %	Extract reducing sugars mg/mL	Extract protein mg/mL
SXXI	T1 (30 min/30°C)	5.20 ± 0.45 b	26.46 ± 0.15 e	9.43 ± 0.28 c
	T2 (120 min/30°C)	5.46 ± 0.51 b	30.76 ± 0.25 d	10.66 ± 0.25 b
	T3 (270 min/30°C)	4.93 ± 0.41 b	34.10 ± 0.34 c	12.26 ± 0.20 a
	T4 (30 min/40°C)	5.76 ± 0.49 b	26.26 ± 0.20 e	9.10 ± 0.17 c
	T5 (120 min/40°C)	6.13 ± 0.81 b	43.20 ± 0.20 b	8.50 ± 0.43 c
	T6 (270 min/40°C)	7.10 ± 0.78 a	46.73 ± 0.15 a	12.96 ± 0.77 a
BTO	T1 (30 min/30°C)	3.46 ± 0.45 d	18.43 ± 0.25 c	12.56 ± 0.05 b
	T2 (120 min/30°C)	4.60 ± 0.10 c	31.23 ± 0.05 a	10.03 ± 0.30 e
	T3 (270 min/30°C)	5.23 ± 0.55 b	24.86 ± 0.15 c	10.86 ± 0.46 d
	T4 (30 min/40°C)	4.33 ± 0.35 c	16.70 ± 0.10 f	9.03 ± 0.57 f
	T5 (120 min/40°C)	5.63 ± 0.15 b	26.76 ± 0.12 b	11.83 ± 0.70 c
	T6 (270 min/40°C)	6.66 ± 0.15 a	20.73 ± 0.16 d	15.83 ± 0.49 a

[a]Values are expressed as mean ± standard deviation ($n = 3$). Different letters within the same column indicate significant differences according to Tukey's test ($p \leq 0.05$).

malts with protein concentrations within this range correlate with higher diastatic power (amylase activity) [33, 34].

The β-amylase activity in the triticale malts was 37.16 BU/g for the SXXI variety and 28.63 BU/g for BTO (Table 1), which represents an increase of 46%–93% compared to the values reported for malts from wheat, rye, barley, maize, and millet (2.61–20 BU/g) [31, 35]. In contrast, rice malt exhibited higher β-amylase activity after 5 and 9 days of germination (60 BU/g and 170 BU/g, respectively) [35]. This variability in amylolytic activity among cereals is consistent with reports in the literature, where such activity is influenced by cereal type, environmental conditions, malting process, and gibberellin production [32]. Recent studies by Bayazitova et al. [36] with the triticale variety Balausa 8 showed an increase in β-amylase activity, reaching 33.32 BU/g after 5 days of germination, suggesting that triticale could serve as an excellent source of β-amylase after the malting process. Furthermore, when combined with α-amylase in brewing worts, triticale could improve the efficiency of maltose generation for beer production [7, 37].

In the present study, α-amylase activity had values of 112.90 ± 0.24 CU/g for BTO malt and 99.10 ± 1.16 CU/g for SXXI malt after 5 days of germination (Table 1), which represented an increase of 17% and 95%, respectively, compared to the values reported for red sorghum, millet, maize, rice, wheat, rye, and barley malts (5–86.83 CU/g) [31, 38]. Farzaneh et al. [22] observed that after 7 days of germination, barley malts could reach α-amylase activity values as high as 290 CU/g. In contrast, Bayazitova et al. [36] reported a value of 217.99 CU/g for triticale Balausa 8 after 5 days of germination. Furthermore, Yu and Liu [6] suggested that triticale exhibited a superior α-amylase production profile in comparison to other cereal species, which is consistent with the results of the present study. This high yield of α-amylase, in conjunction with the β-amylase activity, positions triticale as a promising raw material for enzyme production, with significant applications in both the food and biotechnology industries.

3.2 | Analysis of the Aqueous Extracts

Table 2 shows the results of the total solids, reducing sugars, and protein analyses performed on the aqueous extracts of the triticale malts (varieties SXXI and BTO).

3.2.1 | Total Solids

The total solids content reached its highest values under 40°C/270 min (T6) conditions and its lowest under 30°C/30 min (T1) conditions for the malts of both varieties (SXXI and BTO) (Table 2). In aqueous extracts of plant materials, total solids are primarily derived from sugars generated by amylolytic activity and non-starch saccharides [39]. However, in the present study, negative correlations were observed between total solids content and β-amylase and α-amylase activities (Table 3). This suggests that temperature and agitation time, which exhibited positive correlations (Table 3), may have played a more significant role in increasing total solids content by dissolving non-starch compounds. These findings are consistent with those reported by Duan et al. [40], who indicated that higher total solids production in microbial fermentations does not necessarily correlate with maximum enzymatic activity. This trend was evident in treatment T6, where the highest total solids content was recorded, despite lower α-amylase and β-amylase activities.

3.2.2 | Reducing Sugars

The concentration of reducing sugars showed a variety-dependent response in the malt extracts. In malt extracts from the SXXI triticale variety, the highest value was obtained in T6 (46.73 ± 0.15 mg/mL), with positive correlations regarding both temperature and extraction time (Table 3), suggesting that prolonged and warmer extraction favored carbohydrate solubilization and starch hydrolysis. This behavior is consistent with previous reports suggesting that malting and thermal processing conditions can enhance reducing sugar formation by promoting starch modification and enzymatic accessibility [41]. In contrast, malt extracts from the Bicentenario triticale variety reached their maximum reducing sugar concentration in T2 (31.23 ± 0.05 mg/mL), while temperature was negatively correlated and time showed only a weak positive correlation for the reducing sugars. This finding indicates that moderate extraction conditions were adequate for sugar release in Bicentenario, whereas more severe time–temperature conditions did not further increase reducing sugar accumulation. Such differences may be linked to variety-dependent changes in malt matrix structure, starch accessibility, and endogenous enzyme behavior. Amylolytic activity during cereal germination was strongly influenced by genotype,

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TABLE 3 | Multivariable regression analysis of extracts from the SXXI and BTO varieties.

	Reducing sugars	Total solids	α-Amylase	β-Amylase	Protein	Reducing sugars	Total solids	α-Amylase	β-Amylase	Protein
	SXXI variety					BTO variety				
Reducing sugars		0.6310	−0.8963	−0.8278	0.3974		0.2154	−0.2588	−0.6957	−0.1684
		0.0050	0.0000	0.0000	0.1025		0.3906	0.2998	0.0013	0.5041
Total solids	0.6310		−0.5193	−0.7545	0.2700	0.2154		−0.8856	−0.7324	0.5419
	0.0050		0.0272	0.0003	0.2786	0.3906		0.0000	0.0005	0.0202
α-Amylase	−0.8963	−0.5193		0.7699	−0.5975	−0.2588	−0.8856		0.8252	−0.5904
	0.0000	0.0272		0.0002	0.0088	0.2998	0.0000		0.0000	0.0099
β-Amylase	−0.8278	−0.7545	0.7699		−0.3954	−0.6957	−0.7324	0.8252		−0.5073
	0.0000	0.0003	0.0002		0.1044	0.0013	0.0005	0.0000		0.0316
Protein	0.3974	0.2700	−0.5975	−0.3954		−0.1684	0.5419	−0.5904	−0.5073	
	0.1025	0.2786	0.0088	0.1044		0.5041	0.0202	0.0099	0.0316	
Temperature	0.5264	0.6573	−0.4196	−0.7983	−0.1782	−0.3431	0.5279	−0.2901	0.0253	0.2436
	0.0248	0.0030	0.0830	0.0001	0.4793	0.1633	0.0243	0.2430	0.9206	0.3300
Time	0.6903	0.2460	−0.8536	−0.5306	0.8584	0.3030	0.7748	−0.9135	−0.7916	0.5005
	0.0015	0.3250	0.0000	0.0235	0.0000	0.2216	0.0002	0.0000	0.0001	0.0344

processing conditions, and the biochemical characteristics of the grain [32]. Furthermore, starch hydrolysis in malt systems is not governed only by enzyme activity, but also by substrate accessibility (starch conformation and gelatinization), soluble and insoluble fractions, and processing conditions [42]. Consequently, the different patterns of reducing sugars observed between the triticale malt extracts likely reflect differences in the balance between carbohydrate solubilization, starch hydrolysis, and amylase activity loss during extraction.

3.2.3 | Protein

The protein content in the malt extracts from the BTO variety (9.03 ± 0.57–15.83 ± 0.49 mg/mL) (Table 2) showed positive correlations with both extraction temperature and extraction time (Table 3). In contrast, for the SXXI variety (8.50 ± 0.43–12.96 ± 0.77 mg/mL) (Table 2), only extraction time showed a significant positive correlation (Table 3). These results suggest that longer extraction times enhance protein recovery, although this may have an inverse effect on the enzymatic activities of β and α amylases, as evidenced by the negative correlations observed. Kamal et al. [43] suggested that this phenomenon may be related to the regulation of amylase synthesis by proteolytic enzymes, which also contributes to the solubilization of malt proteins, thus facilitating their extraction. Research on sweet potatoes reported that lower protein concentrations (3.2 mg/mL^{-1}) may result in higher β-amylase activity (55.69 U/mL) [44]. On the other hand, enzymatic extractions using *Abrus precatorius* stems associated higher protein concentrations with greater amylase activity [27]. The lack of a clear pattern of protein concentration suggests the need for further studies to elucidate the specific protein fractions involved in amylolytic enzyme production.

3.2.4 | β-Amylase Activity in Aqueous Extracts From Triticale Malts

Figures 1 and 2 illustrate the β-amylase activity per gram of dry matter (DM). For the BTO variety, activity ranged from 44.73 $\pm$ 0.04 BU/g (T4) to 29.83 $\pm$ 0.05 BU/g (T6) (Figure 1).

In the case of the SXXI variety, the activity ranged from 51.43 $\pm$ 0.50 BU/g (T1) to 41.43 $\pm$ 0.08 BU/g (T6) (Figure 2). Overall,

FIGURE 1 | β-Amylase activity in aqueous extracts from BTO variety triticale malt under T1–T6 treatments. Points represent mean values and error bars indicate standard deviation ($n = 3$). Statistical differences among treatments were evaluated by Tukey's test ($p \leq 0.05$).

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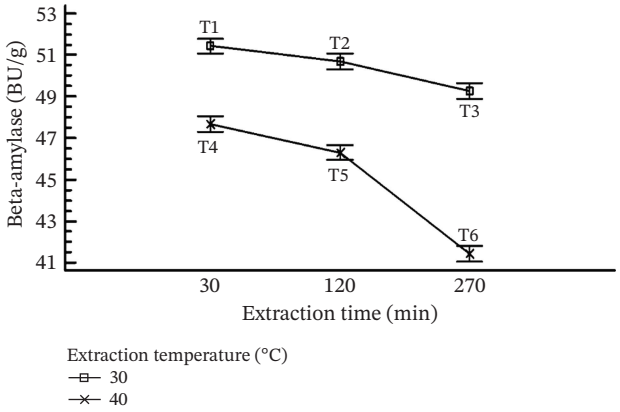


FIGURE 2 | β-Amylase activity in aqueous extracts from SXXI variety triticale malt under T1–T6 treatments. Points represent mean values and error bars indicate standard deviation ($n = 3$). Statistical differences among treatments were evaluated by Tukey's test ($p \leq 0.05$).

the SXXI malt extract exhibited 15% higher ($p \leq 0.05$) enzymatic activity than the BTO variety. In terms of effect magnitude, the highest β-amylase activity observed among the aqueous extraction treatments represented a 1.49-fold increase in BTO and a 1.24-fold increase in SXXI compared with the lowest activity recorded within each variety. This finding indicates that the extraction conditions had a more pronounced effect on the efficiency of β-amylase extraction in BTO, while SXXI showed consistently higher activity across the treatments.

A negative correlation between extraction time and β-amylase activity was observed in malt extracts from both triticale varieties (Table 3), indicating that prolonged extraction did not improve the recovery of active enzyme. Furthermore, a negative correlation was observed between temperature and β-amylase activity in malt extracts from the SXXI variety, whereas Bicentenario showed only a weak and non-significant positive correlation (Table 3). This suggests a variety-dependent response, in which excessive exposure to temperature and time may promote β-amylase destabilization or thermal inactivation rather than further enzyme release. This finding agrees with reports on cereal malts showing significant β-amylase activity losses within 30 min at 73°C, while α-amylase was comparatively more heat stable [45]. In the present study, this trend was reflected in the decrease of β-amylase activity from 44.73 BU/g at T4 to 29.83 BU/g at T6 in BTO (Figure 1), and from 51.43 BU/g at T1 to 41.43 BU/g at T6 in SXXI (Figure 2). Therefore, shorter extraction times and lower temperatures for SXXI may better preserve β-amylase activity while reducing energy input. This behavior may be associated with the sensitivity of β-amylase to processing conditions. For example, recombinant *Bacillus aryabhattai* β-amylase showed maximum activity under defined conditions of pH 6.5°C and 50°C [46]. Moreover, structural studies of microbial β-amylase indicate that substrate recognition depends on specific sugar-binding sites and active-site conformation, highlighting the importance of preserving enzyme structure during extraction and downstream processing [47]. Thus, future optimization should prioritize shorter extraction times, pH control, lower processing severity, and the evaluation of stabilizing conditions to minimize β-amylase activity loss. Table 4 presents the conversion of β-amylase activity from BU/g to BU/mL in the triticale malt extracts. Despite the observed activity, both BTO and SXXI varieties showed lower β-amylase levels in terms of BU/mL compared to those reported for other plant-based materials. For example, Sagu et al. [27] reported 20.42 BU/mL in extracts from *Abrus precatorius* stems, while Hesam et al. [44] reported 55.69 BU/mL in sweet potato extracts. In contrast, the microbial strain *Bacillus aryabhattai* exhibited significantly higher β-amylase activity, ranging from 128.9 to 5371.8 BU/mL, with peak values under optimized conditions [40]. Recent microbial β-amylase studies further confirm that enzymatic performance is highly dependent on strain selection, expression system, and process optimization. For instance, recombinant β-amylases from *Priestia flexa* expressed in *Komagataella phaffii* showed optimal activity at 55°C and pH 8.0, with specific activities of 3411–3836 U/mg [48]. Similarly, immobilized cells of *Bacillus velezensis* AMA2 and *Bacillus atrophaeus* AMA6 increased amylase production from 78 to 232 U/mg and from 71 to 216 U/mg, respectively, while retaining production capacity after repeated reuse cycles [49]. Thus, although the β-amylase activity in triticale malt extracts was lower compared to other plant materials in terms of BU/mL, it was not necessarily inferior when expressed in BU/g, but it remained substantially lower than that of microbial strains. While microbial β-amylase systems typically achieve higher volumetric enzymatic activities, the results obtained from triticale malt should be interpreted within the framework of plant-derived enzyme production. Germinated plant matrices have been reported as relevant sources of amylolytic enzymes with technological potential [50]. In contrast to microbial fermentation, this approach utilizes a renewable cereal matrix, requires simple aqueous extraction, and avoids the use of complex culture media or strict fermentation control. Thus, the value of triticale malt lies not only in enzyme activity, but also in the simplicity of the processing procedure, the reduced processing severity, and potential valorization of residual biomass. These aspects align with sustainable bioprocessing strategies that prioritize simplicity, downstream feasibility, and environmental considerations [51], as well as with the growing interest in plant-derived enzymes as sustainable biocatalysts for food and industrial applications [11].

3.2.5 | α-Amylase Activity in Aqueous Extracts From Triticale Malts

The results of α-amylase activity (DM) are shown in Figures 3 and 4. For the BTO variety, the enzymatic activity ranged from 488.69 ± 1.04 CU/g (T1) to 309.99 ± 1.04 CU/g (T6) (Figure 3).

For the SXXI variety, it varied from 507.41 ± 0.44 CU/g (T1) to 378.81 ± 1.56 CU/g (T6) (Figure 4). In terms of effect magnitude, the highest α-amylase activity observed among the aqueous extraction treatments represented a 1.58-fold increase in BTO and a 1.34-fold increase in SXXI compared with the lowest activity recorded within each variety. These results indicate that the extraction conditions had a clear effect on α-amylase extraction efficiency, with a greater relative response in BTO, although SXXI showed the highest absolute enzymatic activity.

The strong negative correlations between extraction time and α-amylase activity in malt extracts from both triticale varieties (−0.91 for Bicentenario and −0.85 for SXXI) (Table 3) suggest that prolonged extraction favored activity loss rather than additional enzyme recovery. Temperature exhibited a weak negative correlation (Table 3), indicating a potential but less

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TABLE 4 | β-Amylase and α-amylase activities in triticale malt extracts expressed as BU/mL[b] and CU/mL[c], respectively[a].

Treatment	β-Amylase		α-Amylase	
	BU/mL[b] Bicentenario	BU/mL[b] SXXI	CU/mL[c] Bicentenario	CU/mL[c] SXXI
T1 (30 min/30°C)	4.02 ± 0.01 b	5.13 ± 0.05 a	48.87 ± 0.10 a	50.74 ± 0.04 a
T2 (120 min/30°C)	3.25 ± 0.02 c	5.06 ± 0.06 a	45.33 ± 0.09 c	44.16 ± 0.05 c
T3 (270 min/30°C)	3.21 ± 0.01 d	4.90 ± 0.01 b	33.86 ± 0.08 e	41.65 ± 0.02 d
T4 (30 min/40°C)	4.47 ± 0.05 a	4.80 ± 0.01 b	48.45 ± 0.10 b	46.92 ± 0.07 b
T5 (120 min/40°C)	3.11 ± 0.01 e	4.63 ± 0.06 c	36.10 ± 0.06 d	41.27 ± 0.05 e
T6 (270 min/40°C)	2.98 ± 0.01 f	4.10 ± 0.02 d	31.00 ± 0.10 f	37.88 ± 0.16 f

[a]Values are expressed as mean ± standard deviation ($n = 3$). Different letters within the same column indicate significant differences according to Tukey's test ($p \leq 0.05$).
[b]BU/mL: It is defined as the amount of enzyme, in the presence of an excess of thermostable β-glucosidase, required to convert 1 μmol of p-nitrophenol from PNPβ-G3 in 1 min under defined assay conditions.
[c]CU/mL: It is defined as the amount of enzyme, in the presence of an excess of thermostable α-glucosidase, required to convert 1 μmol of p-nitrophenol from BPNPG7 in 1 min under defined assay conditions.

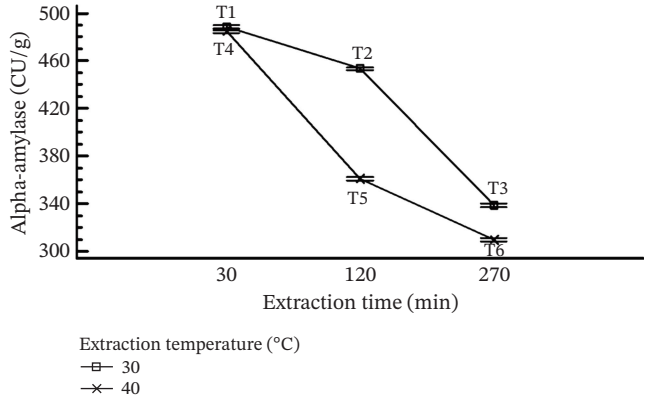


FIGURE 3 | α-Amylase activity in aqueous extracts from BTO variety triticale malt under T1–T6 treatments. Points represent mean values and error bars indicate standard deviation ($n = 3$). Statistical differences among treatments were evaluated by Tukey's test ($p \leq 0.05$).

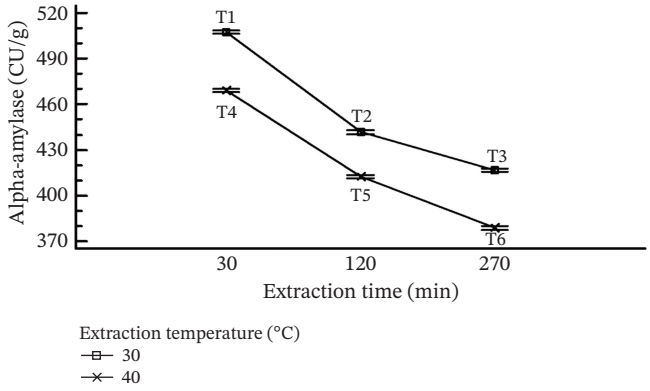


FIGURE 4 | α-Amylase activity in aqueous extracts from SXXI variety triticale malt under T1–T6 treatments. Points represent mean values and error bars indicate standard deviation ($n = 3$). Statistical differences among treatments were evaluated by Tukey's test ($p \leq 0.05$).

pronounced contribution to enzyme destabilization. Although α-amylase is generally more thermostable than β-amylase, its enzymatic activity still depends on maintaining its native conformation within a suitable time–temperature range. Recent evidence in barley malt indicates that α-amylase activity decreases when thermal exposure exceeds its stability range, with genotype-dependent differences in thermostability [52]. Thus, shorter extraction times and moderate temperatures may better preserve α-amylase activity while improving process efficiency. Table 4 presents the conversion of α-amylase activity from CU/g to CU/mL in the aqueous extracts. Compared with the activity reported for *Bacillus* strain BCC 01–50, which reached 4500 CU/mL in an optimized fermentation medium [53], the triticale extracts showed lower volumetric activity. Similarly, Muniasamy and Rathnasaamy [9] reported 2200.13 U/mL using *Bacillus simplex* ON754233 and apple cider pomace, demonstrating the high volumetric productivity achievable through an optimized microbial process. Abd-Elhalim et al. [8] also obtained 695.2 U/mL from *Bacillus amyloliquefaciens* cultivated on bread waste, highlighting the effectiveness of statistically optimized microbial valorization systems. However, when expressed on a dry-matter basis, the SXXI T1 triticale malt extract reached 507.41 ± 0.44 CU/g, a value comparable to the 600 CU/mL reported in an unoptimized microbial medium and higher than the 112 U/mL obtained with *B. amyloliquefaciens* under solid-state fermentation conditions [54]. Likewise, Abo-Kamer et al. [55] reported approximately 250 U/mL from *Bacillus cereus* after statistical optimization, further illustrating the strong influence of culture conditions on microbial α-amylase production. More recently, Adetiloye et al. [56] achieved 196.02 U/mL with *B. cereus* using Box–Behnken optimization, representing a 1.2-fold increase relative to the initial cultivation conditions. Although direct numerical comparisons are limited by differences in activity units, substrates, and assay procedures, these findings place the activity of the triticale malt extract within the range reported for some microbial production systems. Taken together, these comparisons support the use of the selected triticale extracts as amylase-enriched preparations rather than as a direct replacement for highly optimized microbial enzyme concentrates. This distinction is relevant because plant-derived extracts may be more suitable when moderate purification, mild processing conditions, and sustainable sourcing are prioritized over maximum volumetric productivity [10]. The simple aqueous extraction method used in this study may reduce energy consumption and processing time, aligning with sustainable bioprocessing criteria that prioritize simplicity, downstream feasibility, and environmental considerations [51]. In addition, residual malt biomass could be valorized as animal feed or composting

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material, while the subsequent alcohol/salt ATPS is consistent with reports of high enzyme recovery with preserved catalytic activity [13].

Based on the results obtained, the extracts T4 from the BTO variety and T1 from the SXXI variety, which showed the highest enzymatic activity, were selected for enzymatic recovery using ATPS consisting of ethanol and SC.

3.3 | Enzyme Recovery From Aqueous Extracts Using ATPS

The three different ATPS had enzymatic activity in both phases, with statistically significant differences ($p \leq 0.05$) for the activities of α-amylase and β-amylase (Table 5). Under conditions of low ethanol concentration (30%) and high trisodium citrate content (18%), the higher α-amylase activity was concentrated in the lower phase, whereas high values for β-amylase activity were obtained in the upper phase, particularly in variety SXXI (α-amylase 432.89 ± 0.96 CU/mL and β-amylase 5.78 ± 0.05 BU/mL). This phase-dependent distribution suggests that the ethanol/trisodium citrate ATPS promoted differential enzyme partitioning through a multifactorial mechanism governed not only by enzyme hydrophobicity, but also by charge, molecular size, conformation, phase composition, salt type, pH, temperature, and specific enzyme–phase interactions [15, 16].

The values obtained in this research were higher than the α-amylase activity reported for the *Aureobasidium pullulans* fungal strain (120.5 U/mL) in submerged fermentation followed by recovery with ATPS (105.8 U/mL) [57]. In contrast, the β-amylase activity was lower than that reported for aqueous extracts of *Abrus precatorius* stems when the enzyme was recovered via aqueous three-phase systems (32.32 U/mL) [27]. However, recent optimized microbial ATPS platforms have reported substantially higher volumetric activities. For instance, extractive fermentation of *Bacillus simplex* α-amylase using PEG-based deep eutectic solvent ATPS reached 2200.13 U/mL, with 92.4% recovery and a partition coefficient of 3.59 after scale-up to a 3 L bioreactor [9]. Therefore, although the triticale-derived fractions obtained in the present study showed lower volumetric activity than optimized microbial ATPS systems, these comparisons should be interpreted cautiously, since microbial platforms are commonly designed for high volumetric productivity, whereas this approach emphasizes the valorization of a low-cost cereal matrix through mild downstream processing [51].

The superior performance of the 30% ethanol/18% trisodium citrate system indicates that an adequate alcohol–salt balance promoted selective enzyme recovery while minimizing activity loss. This result is consistent with previous reports on alcohol/salt-based ATPS showing high enzyme activity recovery and composition-dependent partitioning [13]. Beyond differential partitioning, these findings support the amylolytic potential of triticale malt, particularly for α-amylase, consistent with previous reports showing that germinated plant matrices can provide enzymes with relevant catalytic and technological properties [50]. Thus, the relevance of triticale malt should also be assessed in terms of renewability, simple aqueous extraction, reduced processing severity, and compatibility with greener purification strategies. In this context, ethanol/trisodium citrate ATPS provides a feasible, water-rich, scalable, and environmentally friendly route for obtaining amylase-enriched fractions from an underutilized crop, although further optimization is needed to improve β-amylase recovery and process scalability [15, 51].

The partitioning parameters of the ATPS are shown in Table 6 and are compared with those reported for ATPS composed of polymers and salts. Shahriari et al. [58] investigated the partitioning of β-amylase in ATPS systems comprising polyethylene glycol and various salts and found partition coefficients for the protein (K_p) ranging from 0.36 to 0.42, indicating that both protein and β-amylase activity partitioned preferentially into the salt-rich phase, with partition coefficients for enzyme activity (K_e) consistently lower than 1. In contrast, in the present study, the K_p values were closer to 1 (Table 6), suggesting an almost equal distribution of protein between both phases. However, β-amylase activity showed a preferential migration toward the ethanol-rich phase, whereas α-amylase was predominantly localized in the SC-rich phase (Table 6), suggesting distinct enzyme–phase affinities between β-amylase and α-amylase [59]. Nevertheless, the comparatively lower β-amylase recovery/

TABLE 5 | Mean values of β-amylase and α-amylase activity in the ATPS composed of ethanol/SC, applied to the T1 extracts from the SXXI variety and T4 extracts from the BTO variety[a].

ATPS		β-Amylase (BU/mL[b])		α-Amylase (CU/mL[c])	
Ethanol (%)	SC[d] (%)				
Top phase		**SXXI**	**BTO**	**SXXI**	**BTO**
45	10	1.56 ± 0.03 e	2.74 ± 0.07 c	22.47 ± 0.09 f	8.33 ± 0.31 f
35	13	5.57 ± 0.05 b	4.14 ± 0.03 b	89.93 ± 0.32 c	48.03 ± 0.35 e
30	18	5.78 ± 0.05 a	5.12 ± 0.03 a	75.30 ± 0.21 d	88.51 ± 0.46 c
Bottom phase					
45	10	1.43 ± 0.02 f	1.03 ± 0.04 e	43.27 ± 0.87 e	67.81 ± 0.24 d
35	13	1.67 ± 0.02 d	0.71 ± 0.02 f	242.05 ± 0.24 b	100.89 ± 0.36 b
30	18	1.78 ± 0.03 c	1.32 ± 0.06 d	432.89 ± 0.96 a	406.25 ± 0.48 a

[a]Values are expressed as mean ± standard deviation ($n = 3$). Different letters within the same column indicate significant differences according to Tukey's test ($p \leq 0.05$).
[b]BU/mL: It is defined as the amount of enzyme, in the presence of an excess of thermostable β-glucosidase, required to convert 1 μmol of p-nitrophenol from PNPβ-G3 in 1 min under defined assay conditions.
[c]CU/mL: It is defined as the amount of enzyme, in the presence of an excess of thermostable α-glucosidase, required to convert 1 μmol of p-nitrophenol from BPNPG7 in 1 min under defined assay conditions.
[d]SC: trisodium citrate.

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TABLE 6 | Partitioning parameters of the two-phase aqueous systems (ATPS) applied to the selected extracts of the SXXI (T1) and BTO (T4) varieties.

ATPS		β-Amylase					α-Amylase				
Ethanol (%)	SC[a] (%)	K_p[b]	K_e[c]	S[d]	F_p[e]	Y (%)[f]	K_p[b]	K_e[c]	S[d]	F_p[e]	Y (%)[f]
SXXI											
45	10	0.78	1.09	1.39	0.27	56.91	0.78	1.93	2.45	0.60	69.94
35	13	0.81	3.33	4.12	0.93	77.69	0.81	2.69	3.32	3.32	73.78
30	18	0.78	3.25	4.18	0.97	81.42	0.77	5.75	7.39	5.69	88.58
BTO											
45	10	0.84	2.65	3.16	0.46	76.31	0.84	8.14	9.73	0.87	90.84
35	13	0.86	5.83	6.77	0.75	81.87	0.86	2.10	2.44	1.46	61.92
30	18	0.91	3.86	4.23	0.76	84.20	0.91	4.59	5.03	5.11	86.37

[a]SC: trisodium citrate.
[b]K_p: protein partitioning coefficient.
[c]K_e: partitioning coefficient of enzyme activity.
[d]S: selectivity.
[e]F_p: purification factor.
[f]Y (%): theoretical yield.

enrichment may be associated with the high susceptibility of this enzyme to the physicochemical microenvironment generated during ATPS partitioning. In ethanol/trisodium citrate systems, ethanol modifies phase polarity and water activity, whereas citrate increases ionic strength and may induce preferential hydration, electrostatic shielding, or salting-out effects. Thus, β-amylase partitioning cannot be explained solely by hydrophobicity, but rather by the combined influence of surface charge, molecular size, conformational stability, exposure of hydrophobic domains, and specific enzyme–phase component interactions. Since β-amylase catalytic performance depends on the preservation of its active-site conformation and substrate-binding regions [47], and ATPS behavior is governed by both protein-intrinsic properties and phase-forming component composition [15], further optimization should focus on ethanol/citrate ratios, pH, phase volume ratio, contact time, temperature, and stabilizing additives to improve β-amylase recovery while minimizing activity loss. This behavior may be attributed to the specific composition of the ATPS. do Nascimento et al. [60] reported that pH, combined with temperature (303.15 and 313.15 K), significantly affected the concentration of α-amylase in the salt-enriched lower phase at pH 5.0, with both K_p and K_e values below 1, or in the PEG-rich upper phase at pH 4.0, 6.0, and 7.0, where both K_p and K_e values exceeded 1.

The yields observed in this study ranged from 56.91% to 84.20% for β-amylase and 61.92%–90.84% for α-amylase (Table 6), which were consistent with the yields reported for the partial purification of amylases from *Bacillus subtilis* C10, *Aureobasidium pullulans*, and *Aspergillus niger* using polymer/salt ATPS systems. α-Amylase preferentially partitioned into the upper phase when the system contained 20%–48% PEG (4000–6000 g/mol) and 7.5%–18% salts such as potassium phosphate, sodium citrate, sodium chloride, lithium sulfate, or sodium sulfate, achieving yields around 59% and 88%. In addition, the Na^+ cation had a significant effect on the size of the biphasic region, increasing the purification factor and partition coefficient [29, 57, 61]. Comparable results have been reported in both biphasic and triphasic aqueous systems for enzyme recovery. Sagu et al. [27] used aqueous triphasic systems to extract β-amylase from *Abrus precatorius* stems, and found that adjustments in pH, the enzyme extract/t-butanol ratio, and ammonium sulfate concentration improved both yield and purification factor. Similarly, in ATPS containing PEG (10%–18%), sodium citrate (12%–20%), and NaCl, used to purify α-amylase from white pitaya peel extracts, partition coefficients and purification factors were optimized under conditions of 14% PEG (6000 g/mol), 16% sodium citrate, and 5% NaCl. In our study, the purification factor was found to increase with the concentration of trisodium citrate (Table 6), probably due to hydrophobic interactions induced by the salt concentration; however, excessive salt concentrations could induce a "salting-out" effect, leading to protein precipitation and consequent loss of enzymatic activity (Shad et al., 2018). In this research, α-amylase was concentrated in the lower phase with increasing SC concentration up to 18%, without significant precipitation in the SXXI and BTO varieties.

Based on the extraction and partitioning results, Table 7 summarizes the optimal conditions identified for α-amylase and β-amylase recovery from triticale malt. This table integrates the main enzymatic responses, selected treatments, ATPS behavior, and technological relevance of the best-performing conditions.

Overall, this summary highlights that mild extraction conditions favored enzyme preservation, while the 30% ethanol/18% trisodium citrate ATPS promoted the most effective differential partitioning of α-amylase and β-amylase. From an industrial perspective, this strategy should be considered a scalable platform for sustainable amylase recovery from triticale malt rather than a final enzyme formulation. The use of an economical cereal matrix, mild aqueous extraction, and ethanol/trisodium citrate ATPS aligns with bioprocessing approaches focused on simplicity, enzyme enrichment, downstream feasibility, and reduced environmental impact [13, 51]. Although ATPS are feasible for scale-up, industrial implementation requires further evaluation of phase separation, reagent consumption, solvent/citrate recovery, mass balance, and process economics [62]. This is relevant because phase-separation purification has been modeled at

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TABLE 7 | Integrated summary of optimal aqueous extraction and ethanol/trisodium citrate ATPS conditions for α-amylase and β-amylase recovery from triticale malt.

Recovery stage	Enzyme fraction	Selected treatment/system	Triticale variety	Enzymatic response	Main interpretation
Aqueous extraction	β-Amylase	30 min/40°C	BTO	44.73 BU/g; 4.47 BU/mL	Highest β-amylase activity in BTO extract
Aqueous extraction	β-Amylase	30 min/30°C	SXXI	51.43 BU/g; 5.13 BU/mL	Highest β-amylase activity in SXXI extract
Aqueous extraction	α-Amylase	30 min/30°C	SXXI	507.41 CU/g; 50.74 CU/mL	Highest α-amylase activity in aqueous extract
ATPS recovery	β-Amylase	30% ethanol/18% trisodium citrate	SXXI	5.78 BU/mL, top phase	Preferential partitioning into ethanol-rich phase
ATPS recovery	α-Amylase	30% ethanol/18% trisodium citrate	SXXI	432.89 CU/mL, bottom phase	Preferential partitioning into citrate-rich phase
ATPS performance	α-Amylase/β-amylase	30% ethanol/18% trisodium citrate	SXXI/BTO	Higher enzyme recovery and enrichment	Best alcohol–salt balance for differential partitioning

production scales of 10–10,000 kg/year, showing potential cost advantages over chromatography at larger scales or with low-purity feedstocks, provided that material use is minimized [14]. In addition, the use of ATPS-purified triticale extracts in reusable amylolytic biocatalysts supports future stabilization, immobilization, and reuse strategies [63].

4 | Conclusion

This study demonstrates the potential of Bicentenario and Siglo XXI triticale malts as renewable cereal matrices for the recovery of amylolytic enzymes, particularly α-amylase. Mild aqueous extraction efficiently recovered both α-amylase and β-amylase, with the best performance achieved at low processing severity, 30 min at 30°C. Subsequent partitioning using ethanol/trisodium citrate ATPS enhanced enzyme enrichment, with the 30% ethanol/18% trisodium citrate system yielding the highest α-amylase activity in the bottom phase, 432.89 CU/mL, and the highest β-amylase activity in the top phase, 5.78 BU/mL, indicating effective differential partitioning while preserving catalytic activity.

Although the recovered activities, especially for β-amylase, were lower than those typically reported for optimized microbial systems, the relevance of this approach lies beyond absolute enzymatic yield. Triticale malt offers a low-cost, underutilized, and renewable matrix that can be processed through simple aqueous extraction and greener downstream strategies. Therefore, ethanol/trisodium citrate ATPS represents a feasible, water-rich, and environmentally friendly route for obtaining amylase-enriched fractions from triticale malt. Overall, this work positions triticale as a sustainable platform for plant-derived amylase recovery, while further optimization of β-amylase recovery, stability, scale-up, and enzyme reuse will be necessary to strengthen its industrial applicability [18, 64].

Author Contributions

Diego Girón-Orozco: conceptualization, methodology, formal analysis, validation, investigation, writing–original draft. Erick Heredia-Olea: conceptualization, supervision, methodology, project administration, writing and editing of the manuscript. María Dolores Mariezcurrena-Berasain: funding acquisition, supervision, visualization, writing–review and editing. Oscar Alejandro Aguilar-Jiménez: methodology, resources, formal analysis, writing–review and editing. Amaury-M. Arzate-Fernández: methodology, resources, investigation.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Data Availability Statement

The datasets generated and analyzed during the current study are publicly available at Mendeley Data under the following DOI: 10.17632/xrpn4t4chc.1 The dataset can be cited as follows: Girón Orozco, Diego., Heredia Olea, Erick., Mariezcurrena Berasain, María Dolores., Aguilar Jiménez, Oscar Alejandro., Arzate Fernández, Amaury Martín. (2025). Data on the Amylolytic Activity of Triticale [dataset]. Mendeley Data, V1. https://doi.org/10.17632/xrpn4t4chc.1.

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