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Detection of diastatic potential in beer by a culture-assisted end-point PCR assay targeting *STA* genes

Detección de potencial diastático en cerveza mediante un ensayo de PCR de punto final asistido por cultivo dirigido a genes *STA*

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Abstract

The presence of diastatic yeast strains in beer poses a recognized spoilage risk, as their ability to hydrolyze dextrins through extracellular glucoamylase activity may lead to secondary fermentation, package instability, and economic losses. Although commercial molecular diagnostic systems are available for brewery quality control, their adoption is often limited by high costs, dependence on proprietary reagents, and logistical constraints in certain regions. In this study, a culture-assisted in-house end-point PCR method was developed and evaluated for the detection of diastatic potential in finished craft beer. The workflow integrated a 48-hour enrichment step in YPD medium, an optimized DNA extraction protocol, and primers targeting conserved regions of the *STA* gene family. Sequence analysis of the amplified product revealed that the assay detects a conserved functional region shared by *STA1*, *STA2*, and *STA2K*, variants associated with a complete glycoside hydrolase family 15 (GH15) domain and functional glucoamylase activity, suggesting a broader detection scope than previously reported for these primers. The method demonstrated 94% accuracy, 100% sensitivity, and 88% specificity under controlled conditions. Compared with a commercial detection system, an overall agreement of 82.9% was observed across finished beer samples. Implementation of this non-proprietary approach reduced consumable costs by 74% and increased thermocycler throughput by 3.2-fold relative to the commercial system. These results suggest that reliable detection of diastatic potential is achievable using standard laboratory infrastructure, offering a technically transferable alternative for brewery quality assurance.

Keywords: beer quality control, diastatic spoilage, starch hydrolysis, molecular diagnostics, technological independence.

Resumen

La presencia de levaduras diastáticas en cerveza representa un riesgo de deterioro reconocido, ya que su capacidad para hidrolizar dextrinas mediante actividad glucoamilasa extracelular puede provocar fermentación secundaria, inestabilidad del envase y pérdidas económicas. Aunque existen sistemas comerciales de diagnóstico molecular para el control de calidad en cervecería, su adopción frecuentemente se ve limitada por altos costos, dependencia de reactivos patentados y restricciones logísticas en ciertas regiones. En este estudio, se desarrolló y evaluó un método interno de PCR de punto final asistido por cultivo para la detección de potencial diastático en cerveza artesanal terminada. El flujo de trabajo integró un paso de enriquecimiento de 48 horas en medio YPD, un protocolo optimizado de extracción de DNA y primers dirigidos a regiones conservadas de la familia génica *STA*. El análisis de secuencia del producto amplificado reveló que el ensayo detecta una región funcional conservada compartida por *STA1*, *STA2* y *STA2K*, variantes asociadas con un dominio GH15 completo y funcional y actividad glucoamilasa, lo que sugiere un alcance de detección más amplio que el reportado previamente para estos primers. El método demostró una exactitud del 94%, una sensibilidad del 100% y una especificidad del 88% bajo condiciones controladas. En comparación con un sistema de detección comercial, se observó una concordancia global del 82.9% en muestras de cerveza terminada. La implementación de este enfoque no patentado redujo los costos de consumibles en un 74% e incrementó el rendimiento del termociclador en 3.2 veces respecto al sistema comercial. Estos resultados sugieren que la detección confiable de potencial diastático es alcanzable utilizando infraestructura de laboratorio estándar, ofreciendo una alternativa técnicamente transferible para el aseguramiento de calidad en cervecería.

Palabras clave: control de calidad de cerveza, deterioro diastásico, hidrólisis de almidón, diagnóstico molecular, independencia tecnológica.

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1 Introduction

The production of bottled and canned beverages is susceptible to contamination by spoilage microorganisms. Although beer presents inhibitory conditions for microbial growth (such as low pH, ethanol content, high carbon dioxide levels, low oxygen availability, and limited simple sugars) contamination of the final product still occurs (Sakamoto & Konings, 2003). Several microorganisms are able to survive under acidic and carbonated conditions and the growth of unwanted yeasts in beer leads to sugar metabolism and the formation of secondary metabolites (Shankar *et al*., 2021; Hellborg & Piškur, 2009). In addition, the excess carbon dioxide production in packaged beer may cause deformation or rupture of containers, resulting in economic losses and potential consumer risk (Suzuki, 2020).

Among beer-spoilage yeasts, wild strains of *Saccharomyces* and species from other genera have been reported, with *Saccharomyces cerevisiae var. diastaticus* (*S. diastaticus*) being one of the most frequently associated with spoilage events (Suzuki, 2020). Contamination by *S. diastaticus* often occurs during bottling and can induce secondary fermentation through the metabolism of residual sugars and dextrins. The diastatic phenotype is primarily associated with the presence of genes from the *STA* family, which encode extracellular glucoamylases (GH15) responsible for dextrin hydrolysis; therefore, detection of conserved regions within these genes is indicative of diastatic potential rather than strict taxonomic identification (Meier-Dörnberg *et al*., 2018). Among the *STA* family variants, *STA1*, *STA2*, and *STA2K* have been shown to encode complete and functional GH15 domains directly associated with glucoamylase activity, whereas other variants such as *STA3* show structural divergence suggesting variable functional contribution (Kim *et al*., 1994).

The relevance of carbohydrate-hydrolyzing enzymatic activity extends beyond spoilage phenomena, since cereal-degrading enzymes are also important in beverage production and other cereal-based biotechnological processes (Vega-Ramírez *et al*., 2026). This process is associated with turbidity, sediment formation, changes in sensory profile, and increased carbon dioxide production that may compromise package integrity (Suiker *et al*., 2021). For this reason, reliable detection of functionally relevant diastatic determinants is an important component of brewery quality control.

Commercial molecular kits are available for the detection of *S. diastaticus*. However, their use in some regions is limited by high costs and logistical constraints, including prolonged lead times from requisition to delivery that hinder the timeliness of quality control. In addition, most commercial systems rely on proprietary formats that offer limited flexibility for methodological adaptation. This type of technological dependence has also been identified in other biotechnological processes, where alternative strategies based on locally available resources have been proposed to reduce reliance on conventional inputs and lower operating costs (Gallardo-Martínez *et al*., 2024). As a result, laboratories with standard molecular biology infrastructure may not fully exploit locally available generic reagents to implement performance-evaluated in-house detection methods.

The objective of this study was to implement and evaluate the performance of a culture-assisted in-house end-point PCR method targeting conserved regions of the *STA* gene family associated with diastatic potential for use in brewery quality control. The analytical performance of the assay under applied conditions was evaluated, and its results were compared with those obtained using a commercial detection system. Sequence analysis of the amplified product revealed that the assay detects a conserved region shared by *STA1*, *STA2*, and *STA2K*, suggesting a broader detection scope than previously reported for these primers. The method demonstrated high sensitivity and accuracy, and its implementation reduced consumable costs substantially relative to the commercial system, supporting its potential as a technically transferable alternative for brewery quality assurance.

2 Materials and methods

2.1 Samples and enrichment strategy

The method was developed and evaluated using two sample types: (i) defined yeast cultures as controls, and (ii) finished, canned, non-pasteurized craft beer samples. These final product samples consisted of non-pasteurized craft beers of varied styles, all obtained from the same production line of Cervecera Hercules. Given the non-pasteurized nature of these products, samples were maintained under refrigerated conditions (4°C) until analysis to preserve their microbiological status. Control strains included *S. diastaticus* (Safale BE-134, positive control) and *Saccharomyces cerevisiae Hansen* (AH22 ATCC, negative control). To ensure detectable levels of diastatic-associated yeast populations, an enrichment step was performed prior to DNA extraction for beer samples.

Beer samples were inoculated by adding 1 mL of beer to 10 mL of sterile YPD broth. For positive and negative controls, pure strains grown on solid medium were aseptically transferred using a sterile

platinum loop into 10 mL of YPD broth. All cultures were incubated at 25 ± 3 °C without agitation. At the end of the incubation period, cells were harvested by centrifugation, and the resulting biomass was used for subsequent DNA extraction and molecular analysis. The incubation time was tested at 24, 48 and 72 h.

2.2 *DNA extraction methods and selection criteria*

Total genomic DNA was extracted from the enriched yeast cultures. Extraction methods were evaluated and compared to select the most suitable protocol for industrial quality control applications: 1) the Edwards buffer method (Edwards *et al*., 1991), 2) the CTAB method (Watanabe *et al*., 2010), 3) a heat lysis method, 4) the commercial InstaGene^{TM} Matrix (Bio-Rad), 5) the modified Edwards protocol (Edwards-2; which is 1) plus isopropanol precipitation, the DNA pellet was washed twice with 70% ethanol to remove residual SDS and salts, followed by air-drying for 10 min). DNA concentration and purity were quantified spectrophotometrically using a Multiskan^{TM} GO, evaluating the absorbance ratios A_{260}/A_{280} and A_{260}/A_{230}.

The integrity and approximate size of the extracted genomic DNA were verified by electrophoresis. For this purpose, DNA samples were loaded onto a 2% (w/v) agarose gel prepared in 1X TAE buffer and stained with 6X Loading Dye and 2 μL of GelRed®. Electrophoresis was conducted at 70 V for 60 min. Gels were visualized and documented using a Gel Doc^{TM} EZ System (Bio-Rad), and images were analyzed with the accompanying ImageLab^{TM} software.

2.3 *End-point PCR and sequencing*

Primers previously reported by Meier-Dörnberg *et al*. (2018) (Forward: 5'-TTCCAACTGCACT AGTTCCTAGAGG-3'; Reverse: 5'-GAGCTGAATGG AGTTGAAGATGG-3') were used. These primers target conserved regions within the *STA* gene family, allowing detection of diastatic-associated genetic determinants rather than strict taxonomic identification. The PCR reaction was assembled as detailed in Table 1. Subsequently, 50-500 ng of the DNA template and the required volume of sterile, nuclease-free water were added to each tube to achieve a final reaction volume of 10 μL. The tubes were then subjected to a brief pulse centrifugation to ensure proper mixing. Amplification was performed in a Techne TC-4000 thermal cycler (Bibby Scientific, UK) with the following program: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 60°C for 30 s, 72°C for 30 s.

Table 1. PCR reaction mix components.

Component	Volume per reaction (μL)
DreamTaq DNA Polymerase (Thermo Scientific)	5.0
Forward Primer (10 μM)	0.6
Reverse Primer (10 μM)	0.6
DNA template	50-500 ng*
Nuclease-free water	(Final volume – 6.2 – DNA volume*)
Final volume	10

*The amount of DNA template must be within a range of 50–500 ng, as recommended by the manufacturer for optimal reaction performance. The volume of the DNA solution added is used to calculate the required volume of nuclease-free water to adjust the final reaction volume to 10 μL.

The expected amplicon size was estimated based on the reference sequence on NCBI accession X02649.1. For visualization, 2 μL of each PCR product were mixed with 2 μL of 6X Loading Dye and 2 μL of GelRed® nucleic acid stain, and subsequently analyzed by 4% agarose gel electrophoresis as previously detailed. PCR products yielding the expected band size were purified and sequenced via the dideoxynucleotide chain-termination method on a 3500 or 3130 Series Genetic Analyzer (Applied Biosystems) at Laboratorio Nacional de Biotecnología Agrícola, Médica y Ambiental (IPICYT). The resulting forward and reverse sequences were assembled, and the consensus sequence was subjected to BLASTn analysis against the NCBI nucleotide database for sequence similarity assessment (Supplementary Figure S3).

2.4 *Bioinformatic analysis of the PCR amplicon and functional domain identification*

The consensus sequence obtained from Sanger sequencing was analyzed using BLASTn against the NCBI nucleotide database to identify sequences with significant similarity to STA family genes associated with diastatic activity. Multiple sequence alignments were performed using the online version of Clustal Omega. Alignments were carried out at both nucleotide and amino acid levels using default parameters. The amplicon sequence was aligned against reference sequences retrieved from NCBI, including *STA1* and the closest matches identified by BLAST analysis. Conserved positions and shared motifs were identified based on the alignment output.

Functional domain analysis was performed using the Conserved Domain Database (CDD, NCBI) online platform. Protein sequences corresponding to *STA1*, *STA2*, *STA2K*, *STA3* and the putative glucoamylase were screened for the presence of the glycoside

hydrolase family 15 (GH15) domain. Domain matches were considered significant when E-values were lower than 1×10^{-5}. Structural visualization and comparison were performed using PyMOL. Predicted protein structures of *STA1*, *STA2* and *STA2K* were analyzed to evaluate the conservation of the GH15 catalytic domain and the spatial distribution of catalytic residues. Structural superposition was used to compare the overall fold of the proteins and to visualize differences in the position of active-site residues. The location of the PCR amplicon within the protein sequence was determined using BLAST-based mapping. Primer-binding sites and the amplified region were positioned relative to the GH15 domain and to conserved catalytic residues, including Glu243 and Glu410, to assess their proximity to functional regions of the protein.

2.5 *Analytical performance metrics*

The analytical performance of the in-house PCR assay was evaluated using known positive and negative control strains over 50 independent replicates each. The standard performance metrics were calculated for a confusion matrix, as defined in Table 2. A confusion matrix was constructed by classifying each replicate result as a true positive (TP), true negative (TN), false positive (FP), or false negative (FN) based on comparison with the known identity of the control strains, and the performance metrics defined in Table 2 were calculated accordingly.

Table 2. Equations for calculating diagnostic performance metrics.

Standard metric	Equation
Accuracy (Acc)	$Acc = \frac{TP+TN}{TP+TN+FP+FN}$
Sensitivity (S)	$S = \frac{TP}{TP+FN}$
Specificity (Sp)	$Sp = \frac{TN}{TN+FP}$
Positive Predictive Value (PPV)	$PPV = \frac{TP}{TP+FP}$
Negative Predictive Value (NPV)	$NPV = \frac{TN}{TN+FN}$

TP: True Positives; TN: True Negatives; FP: False Positives; FN: False Negatives.

All routine sample analyses were performed in technical triplicate. A sample was only considered positive when all three technical replicates showed a clear band of the expected size, and only after the run controls were confirmed as valid.

2.6 *Benchmarking framework*

The analytical performance of the developed in-house PCR method was compared with a commercially available molecular detection kit, hereafter referred to as the commercial kit. The commercial kit employed was BREWSTAT® (bioMérieux S.A.), which utilizes the Veriflow® technology. This system is based on proprietary reagents and a vertical flow cassette-based visualization system, which provides a qualitative (presence/absence) result without the need for electrophoresis.

2.7 *Feasibility of transitioning from commercial kit to in-house method*

A transitional assessment was conducted to define the requirements for migrating from commercial kits to an internally managed diagnostic system. The study focused on how a brewery, already equipped with the baseline infrastructure required for commercial systems, can assume implementation of the *STA1* detection method. The migration analysis was structured into three core components: 1) Infrastructure integration, 2) operational throughput and productivity, 3) supply chain and material procurement.

3 Results and discussion

3.1 *Performance of DNA extraction methods*

Spectrophotometric analysis of the extracted DNA (Figure 1) resulted in variability in both yield and purity parameters across the tested methods. Statistical analysis using one-way ANOVA indicated no significant differences in DNA concentration among the five extraction methods ($F_{4,20}=0.531$, $p=0.714$). As shown in Figure 1a, the mean concentrations ranged from 1007±951 μg/mL for the CTAB method to 1927±1404 μg/mL for the InstaGene method, with all values exceeding the 50 ng template requirement for subsequent PCR analysis.

In contrast to concentration, significant differences were detected in nucleic acid purity as measured by the A_{260}/A_{230} ratio ($F_{4,20}=4.157$, $p=0.013$). Tukey's HSD test revealed that the Edwards-2 method produced DNA with significantly higher A_{260}/A_{230} ratios than the CTAB method ($p=0.026$), indicating superior removal of salt and organic contaminants (Table 3). As illustrated in Figure 1b, the Edwards-2 protocol yielded the most consistent A_{260}/A_{230} values (2.34±0.12), with the lowest variability observed among all protocols (CV = 5.2%). All replicates fell within or near the optimal range of 2.0-2.2 for pure DNA. In contrast, the commercial InstaGene method and CTAB method exhibited the lowest A_{260}/A_{230} ratios (0.88±0.26 and 0.72±0.59, respectively), suggesting potential contamination with phenolic compounds or chaotropic salts.

Table 3. Performance metrics of DNA extraction methods evaluated in this study.

Extraction method	DNA concentration (μg/mL)[1]	A_{260}/A_{230} ratio[1]	A_{260}/A_{280} ratio[1]	CV for A_{260}/A_{230} (%)	Statistical group*
Heat lysis	1754 ± 617	1.95 ± 1.51	1.90 ± 0.40	77.3	ab
Edwards	1800 ± 1626	1.82 ± 0.56	1.92 ± 0.53	30.5	ab
Edwards-2	1348 ± 965	2.34 ± 0.12	2.31 ± 0.02	5.2	b
InstaGene^{TM}	1927 ± 1404	0.88 ± 0.26	1.74 ± 0.35	29.6	ab
CTAB	1007 ± 951	0.72 ± 0.59	2.15 ± 0.30	81.4	a

[1] Values are Mean ± SD, * Methods sharing the same letter do not differ significantly in A260/A230 ratios (n=5, one-way ANOVA followed by Tukey's HSD test, α = 0.05).

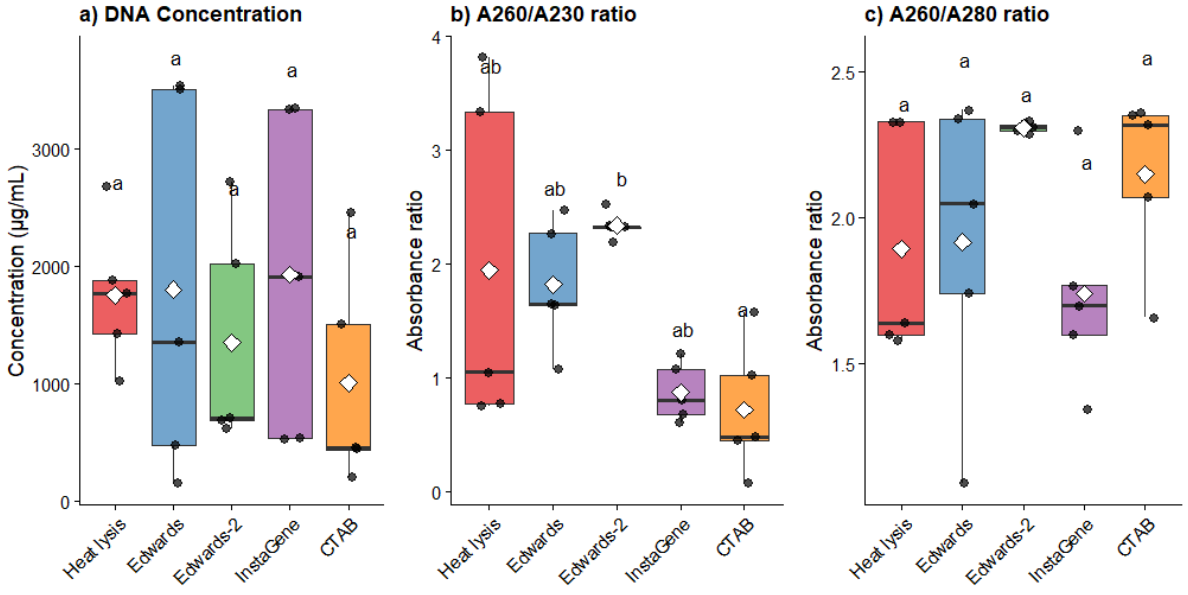

Figure 1. Performance of DNA extraction methods evaluated in this study. a) DNA concentration (μg/mL), b) A_{260}/A_{230} absorbance ratio, and c) A_{260}/A_{280} absorbance ratio obtained using five DNA extraction protocols. Biological replicates n = 5, and diamonds indicate the mean values. Different letters denote statistically significant differences among extraction methods (one-way ANOVA followed by Tukey's HSD test, α = 0.05).

No statistically significant differences were observed in the A_{260}/A_{280} ratios among extraction methods ($F_{4,20}$=1.952, p=0.141), as depicted in Figure 1c. Nevertheless, the Edwards-2 method demonstrated the highest consistency for this parameter (2.31±0.016, CV=0.68%), with minimal variability between replicates. All methods yielded A_{260}/A_{280} ratios within or above the optimal range of 1.8-2.0, indicating generally acceptable protein removal across protocols.

The modified Edwards-2 protocol, which incorporated an additional ethanol washing step following isopropanol precipitation, consistently produced DNA with the most favorable purity profile while maintaining extraction yields comparable to other methods. This enhancement directly addressed purity variability issues commonly associated with traditional extraction protocols, as evidenced by the drastic reduction in CV from 81.4% (CTAB) to 5.2% (Edwards-2), particularly regarding the removal of residual salts and organic contaminants that can interfere with downstream PCR applications (Supplementary Figure S1).

3.2 *PCR amplicon characterization and sequence analysis*

In silico analysis predicted an amplicon size of 79 bp from the *STA1* reference sequence (NCBI accession X02649.1). Amplification of the expected 79 bp fragment was confirmed by sequencing of the purified PCR product through the assembly of overlapping forward and reverse reads (Supplementary Figure S2). Alignment of the resulting consensus sequence with reference STA-family sequences revealed high sequence conservation, supporting the interpretation that the amplified target corresponds to a conserved

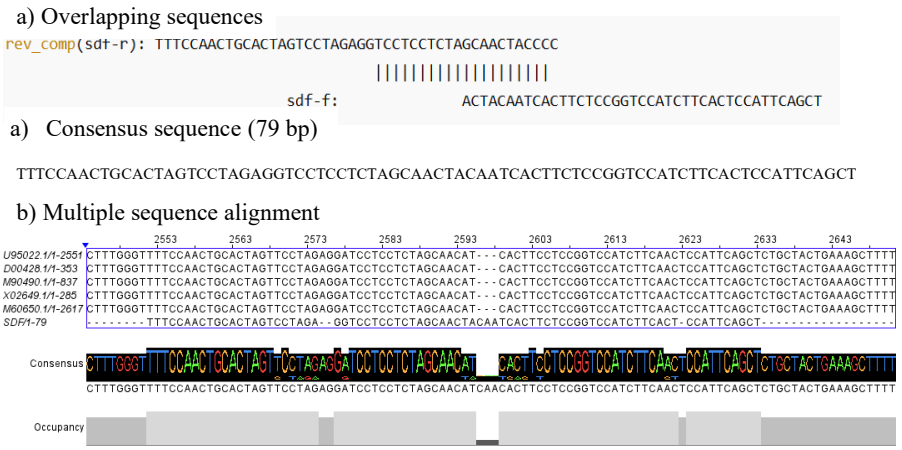

Figure 2. PCR amplicon size confirmation and sequence identity. a) Assembly of forward and reverse sequencing reads showing the overlapping region and the resulting 79 bp consensus sequence obtained from the purified PCR product. b) Multiple sequence alignment of the 79 bp consensus amplicon with reference *STA1*-family sequences.

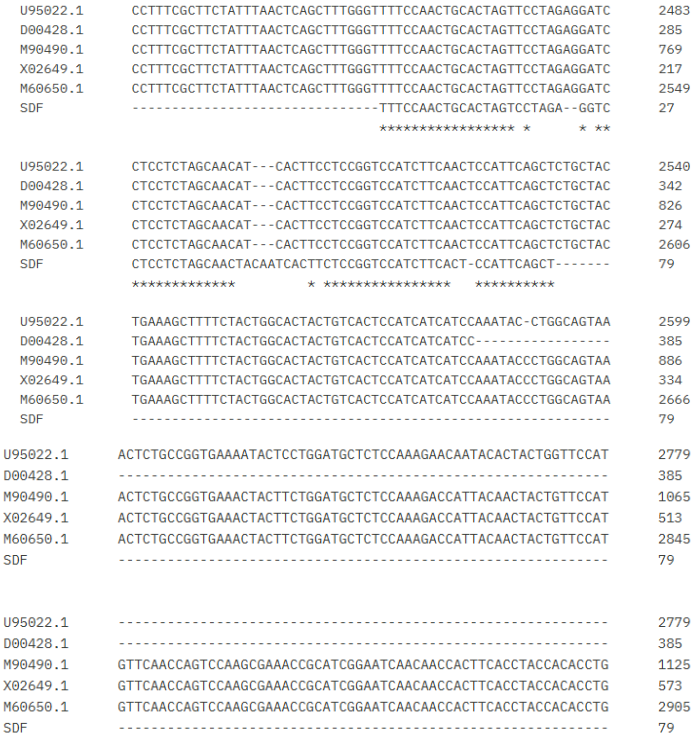

Figure 3. Multiple sequence alignment of the STA gene family. Nucleotide alignment of the consensus amplicon sequence obtained in this study (SDF, derived from the Sdia primer system Sd-f/Sd-r reported by Meier-Dörnberg *et al*., 2018) against reference STA gene sequences. Asterisks () indicate positions with complete conservation.

region associated with diastatic-related glucoamylase genes (Figure 2). Although the primers were originally reported by Meier-Dörnberg *et al*. (2018) as a detection system targeting *STA1*, BLASTn analysis of the amplified sequence revealed significant similarity to additional STA family variants, suggesting a broader detection scope than previously described for these primers.

BLASTn analysis of this consensus sequence against the NCBI database and five sequences producing significant alignments were detected with identity of 90.36% and query cover of 100%: the expected *STA1*, *STA2*, *STA2K*, *STA3* and a putative glucoamylase. The alignment shows that the amplified fragment is located within a region that is highly conserved among these variants, as

Table 4. Functional classification of *STA* variants based on GH15 domain detection.

Variant	GH15 domain detected	Structural conservation	Functional diastatic activity
STA1	Yes (complete)	High	Yes
STA2	Yes (complete)	High	Yes
STA2K	Yes (complete)	High	Yes
STA3	Partial/divergent	Moderate–low	Uncertain / variable
Putative *STA*	Incomplete / absent	Low	Not demonstrated

[1] Conserved Domain Database (CDD, NCBI).

Table 5. Confusion matrix for the in-house end-point PCR assay based on 50 replicates per control strain.

	PCR Positive	PCR Negative
S. diastaticus (positive control)	TP = 50	FN = 0
S. cerevisiae (negative control)	FP = 6	TN = 44

TP: true positives; FN: false negatives; FP: false positives; TN: true negatives.

indicated by the presence of numerous conserved positions across all sequences (Figure 3A). In addition, several conserved blocks are shared specifically among *STA1*, *STA2*, and *STA2K*, whereas *STA3* and the putative sequence display more divergent patterns in this region. This indicates that the amplified fragment corresponds to a core region of the STA gene family shared among major variants associated with diastatic potential. Primer binding analysis further revealed complete sequence conservation at the binding sites for *STA1*, *STA2*, and *STA2K*, while *STA3* and the putative glucoamylase exhibited specific nucleotide mismatches at the 3' end, which may compromise primer annealing and reduce amplification efficiency for these variants. At the amino acid level, *STA1*, *STA2*, and *STA2K* showed high conservation in regions associated with the GH15 domain, while *STA3* presented more pronounced divergence (Supplementary Figure S4).

Because several *STA*-related sequences showed similarity to the amplicon, their association with diastatic potential was evaluated based on the presence of conserved catalytic domains. The glycoside hydrolase family 15 (GH15) domain was identified in *STA1*, *STA2*, and *STA2K*, with highly significant E-values, suggesting that these proteins are likely to encode functional extracellular glucoamylases. This domain is involved in the hydrolysis of dextrins and other polysaccharides into fermentable sugars and is therefore associated with the diastatic phenotype (Vuillemin *et al*., 2024). In contrast, the putative glucoamylase sequence lacked a complete and clearly defined GH15 domain, and no experimental evidence supporting its diastatic activity has been reported. *STA3* showed partial conservation of GH15-related regions but with marked structural divergence, suggesting possible differences in enzymatic activity or regulation (Table 4). Structural superposition of *STA1*, *STA2*, and *STA2K* showed strong conservation of the overall GH15 fold, and the amplified PCR fragment was located within this domain, in proximity to catalytic residues, supporting its functional relevance for detecting diastatic variants.

Taken together, these findings indicate that the assay targets a conserved functional region shared by *STA1*, *STA2*, and *STA2K* — the variants consistently associated with functional glucoamylase activity and diastatic potential in brewing environments. This represents a broader detection scope than initially anticipated based on the primer design reported by Meier-Dörnberg *et al*. (2018), and suggests that the method may provide more comprehensive coverage of functionally relevant diastatic determinants than single-marker *STA1*-based approaches.

3.3 Performance of the End-Point PCR Method

3.3.1 Analytical performance of the in-house PCR assay using control strains

The analytical performance of the PCR assay was assessed using 50 replicates of a known *S. diastaticus* (positive control) and 50 replicates of a *S. cerevisiae* non-diastaticus strain (negative control). The observed results are summarized in a confusion matrix (Table 5): 50 true positives (TP), 44 true negatives (TN), 6 false positives (FP), and 0 false negatives (FN). Amplicons from both the positive control strain and a representative positive beer sample were confirmed by Sanger sequencing, verifying that the amplified product corresponds to the expected target region within the *STA* gene family, consistent with the sequence analysis reported in Section 3.3. The method demonstrated high accuracy (94%), sensitivity (100%), and good specificity (88%) (Figure 4).

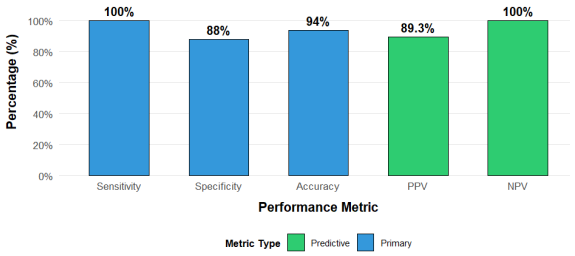

Figure 4. Analytical performance metrics of the in-house end-point PCR method for *S. diastaticus* detection. Error bars were not applicable as values represent percentages calculated from population totals.

The six false positives observed in the negative control were attributed to potential cross-contamination as commonly reported in routine PCR workflows during pipetting, a common challenge in molecular workflows. To mitigate the risk of cross-contamination inherent in manual pipetting, the workflow requires that all samples be analyzed in technical triplicates. A positive result is only reported when all three replicates show consistent amplification. This decision-rule effectively raises the operational specificity of the diagnostic process, ensuring that sporadic contamination during sample handling does not lead to unnecessary product rejection or costly interventions.

These metrics confirm the method's inherent reliability for distinguishing *S. diastaticus* from standard brewer's yeast under controlled conditions. The specificity value indicates a low false-positive rate, which can be further reduced by strict adherence to Good Laboratory Practices and by implementing a triplicate testing scheme with run-validation criteria (e.g., invalidating runs if the negative control shows amplification). The performance of this assay was evaluated against defined control strains under controlled conditions; its applicability across diverse beer matrices warrants further investigation. While kits claim specificity for *S. diastaticus*, their exact genetic target and coverage of the *STA* family are often not disclosed. This method provides transparent and verifiable specificity for the diastatic determinants targeted by the assay.

3.3.2 Comparison with a Commercial Detection System in Finished Beer Samples

To benchmark the in-house PCR method under realistic and stringent conditions, a concordance study was performed using finished, canned, non-pasteurized craft beer samples. This matrix represents a high-risk category for refermentation and spoilage, and its analysis under preserved cold-chain conditions supports the evaluation of the method's applicability in routine industrial quality control.

The commercial kit employed in this study specifies a 48-hour enrichment period in its recommended protocol. To evaluate whether the in-house method could achieve reliable detection at a shorter enrichment time, a subset of the samples confirmed positive in this concordance study was subjected to enrichment at 24, 48, and 72 hours. No amplification was observed at 24 hours across all samples tested, whereas consistent amplification was obtained at both 48 and 72 hours, establishing 48 hours as the minimum operational enrichment time, consistent with the commercial protocol (Supplementary Table S1). At 48 hours of enrichment, a cell concentration of $4.56 \pm 0.35 \times 10^7$ cells/mL was recorded (Supplementary Table S2). Although this does not constitute a formal limit of detection, it provides a reference for the biomass level at which consistent amplification was achieved under the tested conditions, representing a practical and reproducible operational parameter for industrial implementation.

Each analytical run of the in-house assay included positive and negative DNA controls, with a positive result reported only when all three technical replicates showed consistent amplification and run controls performed as expected. This run-validation criterion provides a built-in quality assurance layer that is not offered by the commercial kit, which provides a single qualitative result per sample without replication or declared internal controls.

The analysis revealed an overall agreement of 82.9% (29/35) between the two methods. The six samples that were positive only by the commercial kit could not be independently verified in the absence of an independent reference method. One possible explanation is that the commercial kit may be detecting DNA from non-viable cells or genetic traces below the threshold of technological risk, given that conventional PCR detects DNA regardless of cell viability. The absence of physical spoilage signs in the six discordant samples during shelf-life monitoring is consistent with this interpretation, though independent confirmation would be required to draw definitive conclusions.

3.4 Feasibility of transitioning from commercial kit to in-house method

3.4.1 Infrastructure reclamation and asset optimization

The feasibility of transitioning to the in-house method was evaluated based on the reclamation of existing high-value assets. The baseline infrastructure required for the commercial system, specifically the conventional thermocycler, serves as the primary platform for the proposed protocol. While the commercial system is limited by a reliance on proprietary reagents and a closed visualization

device, the in-house method was found to offer greater methodological flexibility through minor equipment additions. The incorporation of a benchtop microcentrifuge (estimated at USD 50, e-shop) and an electrophoresis system was identified as the sole incremental requirement. Two implementation scenarios were considered: a high-investment conventional system (USD 4,400 by specialized supplier) and a compact, integrative alternative such as the blueGel^{TM} unit (USD 309, e-shop), which facilitates a more accessible transition for smaller-scale industrial laboratories.

3.4.2 *Operational throughput and equipment productivity*

A significant technical advantage regarding thermocycler occupancy was identified. The proposed in-house PCR protocol occupies the equipment for approximately 75 minutes. In contrast, the commercial system requires a total commitment of the thermocycler for 240 minutes due to its rigid pre-set programs (Digest and PCR stages). This represents a 3.2-fold increase in operational throughput, specifically referring to the capacity of the primary asset to process sequential batches. This optimization allows the laboratory to effectively triple its daily analytical capacity without additional capital expenditure in thermal cycling infrastructure.

3.4.3 *Economic impact and supply chain sustainability*

The cost of consumables for the in-house method was estimated at USD 14.63 per test, compared to USD 55.64 for the commercial kit, representing a 74% cost reduction (Figure 5). This reduction is consistent with cost differentials reported for in-house primer-based approaches relative to commercial kits in brewery molecular diagnostics (Oldham and Held, 2023). This shift toward generic reagents (primers, standard polymerases, and nucleotides) is expected to eliminate risks associated with price volatility, import delays, and the potential discontinuation of single-supplier proprietary components.

Comparable cost-rationalization logic has also been documented in other biotechnological processes, where the replacement of commercial inputs by native biological systems reduced external dependence. An analogue example is proposed by Garza-Garza *et al*. (2025), their strategy enabled a biotechnological process such as hydrolysis with no commercial reagents or enzymes. In this way, internalizing the procurement of standard molecular biology reagents, a higher testing frequency can be supported without imposing additional financial burdens on the brewery's quality assurance budget.

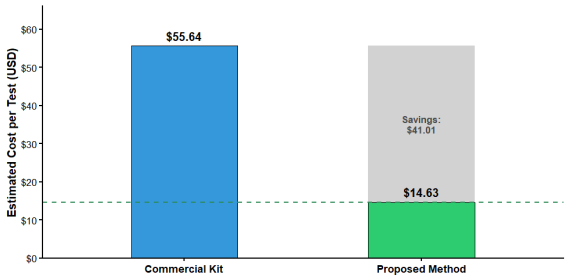

Figure 5. Estimated cost per test (USD) for molecular detection of *Saccharomyces cerevisiae var. diastaticus* under general laboratory conditions. The dashed line indicates the consumable cost associated with the proposed method. Cost estimation includes molecular biology and microbiology reagents and consumables but excludes personnel costs and baseline infrastructure common to both approaches.

3.4.4 *Diagnostic confidence and methodological versatility*

The transition to an in-house system was found to strengthen diagnostic confidence through the explicit use of positive and negative controls in every run. Unlike the no-declared internal controls of proprietary systems, this approach ensures full traceability of results. Furthermore, the established infrastructure was found to be highly adaptable; by replacing enrichment culture medium and specific primers, the same workflow can be utilized for the detection of other relevant spoilage microorganisms, such as *Pectinatus* spp. and *Megasphaera* spp. This versatility aligns the brewery's quality control with engineering principles of robustness and scalability, enabling a shift from sporadic end-product testing to a proactive, in-process diagnostic strategy.

More sensitive quantitative molecular technologies, such as quantitative PCR (qPCR), offer advantages over end-point PCR in terms of detection sensitivity and quantification capability. In particular, qPCR enables the detection of very small microbial populations in brewery samples and is currently considered the method that best meets the desired characteristics for routine monitoring in craft breweries (Oldham and Held, 2023). However, its implementation requires dedicated fluorescence-based instrumentation that represents a substantial capital investment beyond the conventional thermocycler already present in facilities using commercial kit-based detection systems. For breweries where the primary operational goal is reliable qualitative detection of diastatic potential within an existing infrastructure, end-point PCR represents a practical and immediately deployable alternative.

Conclusions

This study developed and evaluated an in-house end-point PCR assay for the detection of diastatic potential in craft beer, providing a technically transferable alternative to commercial systems. Through the optimization of DNA extraction protocol suitable purity was obtained, ensuring the reliability of downstream applications. Bioinformatic analysis and structural modeling of the GH15 domain suggested that the assay targets a highly conserved functional region of the STA gene family. Notably, sequence analysis revealed that the amplified region is shared by *STA1*, *STA2*, and *STA2K*, variants consistently associated with functional glucoamylase activity and diastatic potential, suggesting a broader detection scope than initially anticipated based on the primer design reported by Meier-Dörnberg *et al*. (2018).

The analytical evaluation demonstrated favorable performance with 94% accuracy, 100% sensitivity, and 88% specificity under controlled conditions. Furthermore, the implementation of this method within an industrial framework proved to be highly efficient, reducing consumable costs by 74% and increasing equipment throughput through the optimization of thermocycler occupancy. These results suggest that the detection of diastatic potential in brewery quality assurance is achievable using standard infrastructure and generic reagents.

Despite the operational advantages, certain limitations must be acknowledged. The method was evaluated using a single non-pasteurized craft beer matrix obtained from one production line; its applicability across beer styles with varying composition, alcohol content, and filtration status warrants further investigation. Additionally, the absolute analytical limit of detection in terms of Colony Forming Units (CFU/mL) was not determined, as this study prioritized operational feasibility over absolute quantification. While the 48-hour enrichment was identified as a practical proxy for detectability, the exact minimum biomass required for a positive signal remains to be quantified. Future investigations should focus on establishing the limit of detection across diverse and complex beer matrices. Additionally, the development of rapid sample concentration techniques, such as membrane filtration or selective centrifugation, could be explored to further reduce the total time-to-result. These improvements would facilitate the evolution of the current method into an even more rapid diagnostic tool for the brewing industry.

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Use of artificial intelligence tools

Artificial intelligence tools, including ChatGPT (OpenAI) and Claude (Anthropic), were used to assist with language editing and sentence structure. All scientific content, experimental data, interpretations, and conclusions were developed exclusively by the authors.

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