STUDIA UBB CHEMIA, LXXI, 2, 2026 (p. 7-20)
(RECOMMENDED CITATION)
DOI:10.24193/subbchem.2026.2.01

PARAMETRIC STUDY AND OPTIMIZATION OF D-GLUCOSE ISOMERIZATION USING SWEETZYME IT: A FACTORIAL DESIGN APPROACH

Souad-Diana TORK[a]*, Mălina Ioana POP[a], Alina FILIP[a], Judith-Hajnal BARTHA-VARI[a]

ABSTRACT. High-fructose corn syrup (HFCS) is produced industrially by the immobilized glucose isomerase Sweetzyme IT (from *Streptomyces murinus*), one of the most widely used biocatalysts in the food industry. Although its individual operating parameters are well established, simultaneous interactions between operational variables over time have rarely been systematically quantified. In this study, a two-level full factorial design modeled the combined effects of initial glucose concentration, temperature and Mg^{2+} cofactor concentration on the process. Reaction progress was followed polarimetrically through a dedicated glucose-fructose calibration; concurrent refractometric monitoring proved insensitive due to total dissolved solids conservation. The regression models ($R^2 > 0.99$) identified temperature as the dominant operational factor. Incorporating reaction time as a factor revealed a significant temperature × time interaction and a shift of rate control from initial substrate concentration to temperature near equilibrium. A maximum conversion of 45.98 % (close to the thermodynamic equilibrium of the reaction) was obtained at 0.2 M glucose, 60 °C and 1.5 mM Mg^{2+} after 24 h. A comparative screening of divalent cations confirmed $MgSO_4 \cdot 7H_2O$ as the superior chemical activator, over Ni^{2+}, Mn^{2+}, Cu^{2+} and Ca^{2+} with Ca^{2+} among the poorest, consistent with its known inhibitory role. This study establishes a reliable mathematical approach that can be extended to predict and optimize other complex bioprocesses.

Keywords: *enzymatic isomerisation; HFCS; Sweetzyme IT; glucose isomerase; design of experiments / factorial design; polarimetry; optical rotation; D-glucose*

[a] *Babeş-Bolyai University, Faculty of Chemistry and Chemical Engineering, 11 Arany Janos str., RO-400028, Cluj-Napoca, Romania*
* *Corresponding author: souad.tork@ubbcluj.ro*

INTRODUCTION

High-fructose corn syrup (HFCS) has become an essential component in the food and beverage industry, representing an economical alternative to traditional sucrose. [1, 2] The major industrial interest in this product is based on the superior properties of fructose, which is the sweetest naturally available carbohydrate (being approximately 1.7 times sweeter than glucose) making it particularly valuable for dietary products and processed foods. [3] The large-scale production of HFCS is achieved through the interconversion of D-glucose to D-fructose, a process that can be approached both chemically and biocatalytically. [4-6]

Although the isomerization reaction can be chemically catalysed in alkaline or acidic media, chemo-catalytic processes present major technological disadvantages: they require high temperatures and pH values, have low selectivity (typically yielding 20-30%), and generate non-metabolizable by-products (e.g., psicose) or degradation compounds that alter the colour and flavour of the final product. [3, 7] For these reasons, enzymatic processes mediated by glucose isomerase (GI) (D-xylose ketol-isomerase, EC 5.3.1.5) have become the undisputed industrial standard, offering exceptional reaction specificity, operation under mild ambient conditions, and generating no unwanted by-products. [6, 8]

Glucose isomerase is a metalloenzyme whose catalytic activity and structural stability depend strictly on the presence of divalent metal cations, such as Mg^{2+}, Co^{2+}, or Mn^{2+}. Advanced structural studies, including X-ray and neutron diffraction, have demonstrated that the enzyme possesses two distinct metal sites (M1 and M2) that orchestrate the catalytic mechanism (Figure 1A). [9-10] The generally accepted reaction mechanism proceeds in three fundamental steps: the opening of the sugar ring, an isomerization step mediated by metal ions via a hydride transfer (from C2 to C1), and finally, ring closure to release the fructose (Figure 1B). [9, 11]

Despite its high efficiency, GI is predominantly an intracellular enzyme, and its use in a free, soluble form involves major losses and prohibitive costs for continuous industrial processes. [3] To overcome these limitations, the established approach has consisted of immobilizing the enzyme on solid supports, an approach that facilitates the recovery and repeated reuse of the biocatalyst while simultaneously solving issues of thermal and operational stability. [12] In particular, commercial preparations such as Sweetzyme IT (Novozymes) are widely used due to their robustness in continuous-flow packed-bed reactors. Derived from a selected strain of *Streptomyces murinus*, this specific biocatalyst is obtained through the glutaraldehyde cross-linking of whole-cell homogenates combined with an inorganic carrier. Engineered

into dense, cylindrical particles (0.2–0.4 mm in diameter) with a high specific activity (e.g. 450 IGIU/g), Sweetzyme IT exhibits exceptional mechanical and catalytic stability, making it highly effective for the large-scale industrial conversion of glucose to fructose. [13-15]

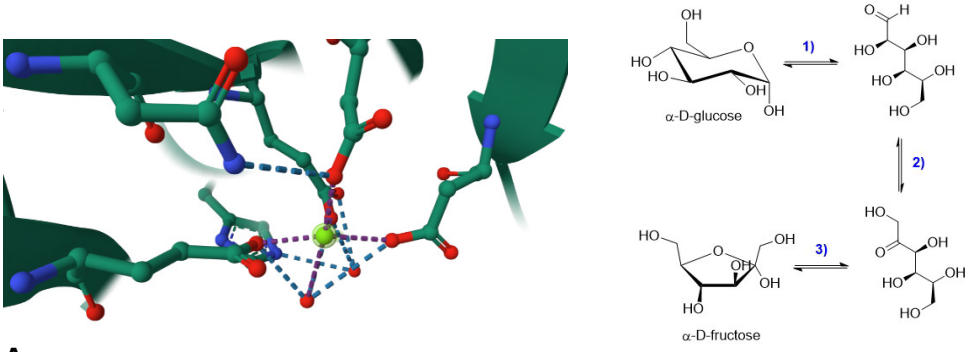

Figure 1. A) M2 catalytic site of *Streptomyces murinus* glucose isomerase (PDB ID: 1DXI). The catalytic magnesium ion (Mg^{2+}) is tightly coordinated by conserved residues His220, Glu217, Asp257, and Asp255 (bidentate binding). Purple dashed lines represent direct metal-ligand coordination, while blue dashed lines indicate hydrogen-bonding networks; **B)** Reaction mechanism of D-glucose to D-fructose interconversion. The process proceeds through three fundamental steps: 1) ring opening of α-D-glucose, 2) metal-mediated isomerization via hydride transfer, and 3) ring closure to yield α-D-fructose.

The aim of this study is to evaluate and optimize the kinetics and efficiency of the enzymatic isomerization of D-glucose to D-fructose using the commercial immobilized biocatalyst Sweetzyme IT. To understand the impact of the reaction parameters, a factorial design was employed to investigate key operational conditions (initial glucose concentration, temperature and magnesium cofactor concentration) and the activating or inhibiting effects of alternative metal cations on the enzyme's specific activity were evaluated.

Glucose isomerase is among the most thoroughly studied industrial enzymes, and the behaviour of commercial preparations such as Sweetzyme IT is well documented at the level of individual operating parameters. The contribution of the present study therefore lies not in the enzyme or the reaction themselves, but in how their operational space is modelled and monitored. First, in contrast to the prevailing one-factor-at-a-time practice, a two-level full factorial design quantifies the interactions among substrate concentration, temperature and cofactor concentration for this specific

commercial biocatalyst, yielding a predictive regression model ($R^2 > 0.99$) rather than a set of isolated optimal values [16-18]. Second, by treating reaction time as an explicit factor, the analysis reveals that the rate-controlling variable changes during the reaction (substrate-controlled at short times and temperature-controlled near equilibrium, with a statistically significant temperature × time interaction) a kinetic feature that conventional single-time-point optimisation cannot capture. Third, the work provides a validated, low-cost polarimetric assay that converts a single optical-rotation reading into conversion, and shows that refractometry, although standard for sugar syrups, is intrinsically blind to this mass-conserving isomerisation. Together, these establish a compact, reproducible design-and-monitor methodology that is readily transferable to other immobilised glucose isomerases and to process-scale decisions [19, 20].

RESULTS AND DISCUSSION

The conversion of D-glucose into D-fructose catalysed by Sweetzyme IT was first quantified by a dedicated polarimetric calibration (Section 1) and then optimised through a two-level full factorial design (Section 2). The three operational variables - glucose concentration, temperature and Mg^{2+} concentration - were modelled as a 2^3 design (Section 2.1), while the role of reaction time was resolved by treating it as a fourth factor in a 2^4 analysis (Section 2.2). Finally, the activating/inhibiting effect of alternative divalent cations was screened (Section 3).

1. Polarimetric calibration and determination of conversion

D-glucose and D-fructose are both optically active but rotate plane-polarised light in opposite directions and the optical rotation of a reaction mixture is a direct, non-destructive proxy for its composition. Eleven standard glucose-fructose mixtures (total 1 M, 0.01 M $MgSO_4 \cdot 7H_2O$, 100 mm cell, 589 nm, 25 °C) were measured to construct the calibration curve (Figure 2). Pure glucose is strongly dextrorotatory ($\alpha_G = +51.22°$) and pure fructose strongly levorotatory ($\alpha_F = -80.69°$); the response is linear over the whole composition range:

$$\alpha = 50.44 - 131.15 \times X_F \quad (R^2 = 0.9999) \quad \text{(Eq. 1)}$$

Since optical rotation is additive, the residual glucose concentration in any sample of total monosaccharide concentration (C_{total}) is obtained from

$$C_G = (\alpha - C_{total} \times \alpha_F) / (\alpha_G - \alpha_F);\ C_F = C_{total} - C_G \quad \text{(Eq. 2)}$$

and the molar conversion of glucose into fructose is

$$\text{Conversion (\%)} = C_F / C_{total} \times 100 \quad \text{(Eq. 3)}$$

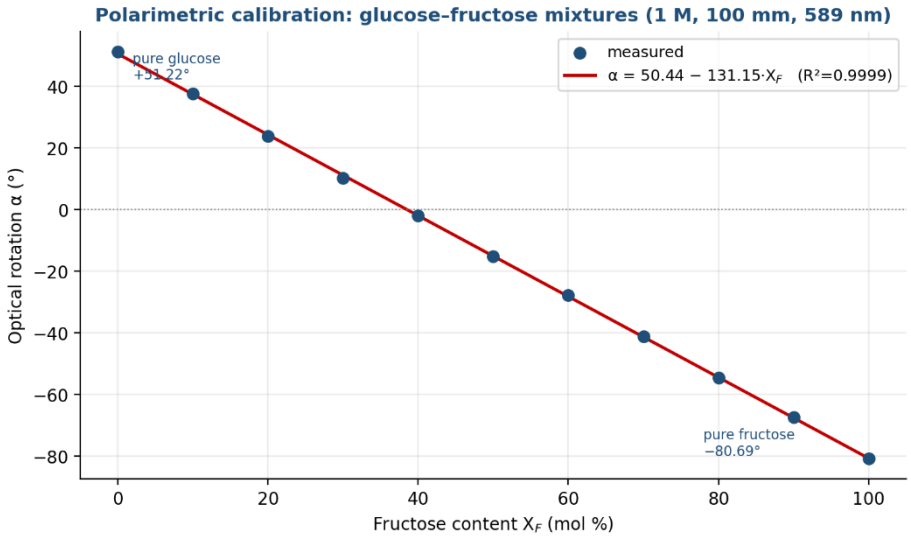

Figure 2. Polarimetric calibration of glucose-fructose mixtures. The linear fit (Eq. 1) is used with Eqs. 2–3 to convert every measured optical rotation into a conversion value.

2. Factorial-design optimization of the isomerization

The optimization of reaction conditions is a critical step in any bioprocess, aiming to maximise conversion while minimising time, energy and material costs. Traditional one-factor-at-a-time (OFAT) optimization is laborious and fails to capture the interactions between variables. Design of Experiments (DoE) offers a more powerful, systematic alternative: a two-level full factorial design allows the simultaneous investigation of several variables, quantifying both their main effects and their interaction effects, and fits the data to a mathematical model that defines the input–response relationship over the experimental region [16, 18].

2.1 Operational factors: a 2^3 full factorial design (response: conversion %)

Three operational variables were selected as the factors most likely to govern the isomerization: initial glucose concentration (z_1), temperature (z_2) and Mg^{2+} concentration (z_3). Each was studied at two coded levels, −1 (low) and +1 (high), about a central point (z_{10} = 0.4 M, z_{20} = 50 °C, z_{30} = 1.0 mM), giving a 2^3 = 8-run design (Table 1). The reaction was monitored polarimetrically and the optical rotation converted to conversion via Eq. 1–3; the full 16-point data set (eight conditions sampled at 4 h and 24 h) is given in Table 2**.**

Table 1. Experimental factors and their coded levels utilized in the factorial design matrix.

Factor	Symbol	Units	Low (−1)	Centre (0)	High (+1)
[glucose]	Z_1 (A)	M	0.2	0.4	0.6
Temperature	Z_2 (B)	°C	40	50	60
$[Mg^{2+}]$	Z_3 (C)	mM	0.5	1.0	1.5

Table 2. Experimental matrix, measured optical rotation and derived conversion.

Run	[G] (M)	T (°C)	$[Mg^{2+}]$ (mM)	Time (h)	α (°)	C (%)
1	0.6	60	1.5	4	3.120	34.88
2	0.2	60	1.5	4	1.833	31.88
3	0.6	40	1.5	4	4.176	33.55
4	0.2	40	1.5	4	4.194	22.93
5	0.6	60	0.5	4	2.657	35.47
6	0.2	60	0.5	4	2.167	30.61
7	0.6	40	0.5	4	4.380	33.29
8	0.2	40	0.5	4	4.333	22.40
9	0.6	60	1.5	24	-1.528	40.76
10	0.2	60	1.5	24	-1.889	45.99
11	0.6	40	1.5	24	2.148	36.11
12	0.2	40	1.5	24	0.944	35.25
13	0.6	60	0.5	24	-1.454	40.66
14	0.2	60	0.5	24	-1.556	44.72
15	0.6	40	0.5	24	2.231	36.01
16	0.2	40	0.5	24	2.028	31.14

Considering the 24 h end-point, temperature is by far the dominant operational factor: it accounts for 78 % of the variance in conversion, raising it on average by +8.4 percentage points between 40 °C and 60 °C. The glucose concentration × temperature interaction (AB) is the next largest contribution, while the main effects of Mg^{2+} (+1.4 pp) and glucose concentration (-0.9 pp) are minor. The first-order model in coded factors is:

$$C\ (\%) = 17.33 - 2.22\times [G] + 0.420\times T + 1.39\times [Mg^{2+}] \quad \text{(Eq. 4)}$$

The positive temperature coefficient confirms that, within the 40-60 °C interval, raising the temperature accelerates the approach to the fructose-rich equilibrium. The minor positive Mg2+ term indicates that even the low level (0.5 mM) nearly saturates the enzyme's metal sites, so additional cofactor yields little extra conversion. Interestingly, at the early (4 h) sampling point the ranking is reversed: glucose concentration dominates (+7.3 pp) ahead of temperature (+5.2 pp), reflecting a substrate-driven initial rate before the system approaches equilibrium. This time-dependent shift in factor importance motivates the time-resolved analysis. The highest experimental conversion, 45.98 %, was obtained at 0.2 M glucose, 60 °C, 1.5 mM Mg^{2+} after 24 h (Run 10, Table 2), close to the ~45 % thermodynamic ceiling of the reversible isomerization.

2.2 Resolving reaction time: a 2^4 analysis (response: conversion %)

To quantify the role of reaction time and its coupling with the operational factors, the two sampling times (4 h and 24 h) were incorporated as a fourth two-level factor (D), expanding the design to a 2^4 = 16-run experiment. A first-order model with two-way interactions was fitted; the third- and fourth-order interactions (5 degrees of freedom) provided the error estimate. The ANOVA (Table 3) shows a highly significant model, and the model summary (Table 4) confirms an excellent and well-balanced fit.

Table 3. ANOVA for the 2^4 design (response = conversion %). Terms with P ≤ 0.05 are significant; the dominant terms are highlighted.

Source	DF	Adj SS	Adj MS	F	P
Model	10	632.68	63.27	126.93	0.000
D (Time)	1	269.12	269.12	539.92	0.000
B (Temp.)	1	184.24	184.24	369.62	0.000
AD	1	67.78	67.78	135.98	0.000
AB	1	51.38	51.38	103.07	0.000
A ([G])	1	41.65	41.65	83.56	0.000
BD	1	10.49	10.49	21.04	0.006
AC	1	3.32	3.32	6.67	0.049
C ([Mg^{2+}])	1	3.09	3.09	6.21	0.055
CD	1	1.05	1.05	2.11	0.206
BC	1	0.55	0.55	1.10	0.342
Error	5	2.49	0.498		
Total	15	635.17			

Table 4. Model summary statistics for the 2^4 model.

S	R^2	R^2 (adj)	R^2 (pred)
0.706	99.61 %	98.82 %	95.98 %

Reaction time (D) emerges as the single most influential variable (F = 540, P < 0.001), narrowly ahead of temperature (B; F = 370, P < 0.001). The two factors that couple with time and substrate concentration (the glucose concentration × time (AD) and glucose concentration × temperature (AB) interactions) are also highly significant, as is the temperature × time interaction (BD; F = 21.0, P = 0.006). The reduced model in coded factors is:

$$C\ (\%) = 34.73 + 1.61 \times A + 3.39 \times B + 0.44 \times C + 4.10 \times D - 1.79 \times AB - 0.46 \times AC - 2.06 \times AD + 0.81 \times BD \quad \text{(Eq. 5)}$$

The Pareto chart (Figure 3) confirms this ranking: the bars for D, B, AD, AB, A, BD and (marginally) AC extend beyond the t = 2.571 significance reference line, whereas the Mg^{2+} main effect (C) sits just below it (P = 0.055). The temperature × time interaction is best appreciated in the response surface (Figure 4) and the interaction plot (Figure 5): the two temperature lines are not parallel, the benefit of operating at 60 °C is modest at 4 h (+5.2 pp over 40 °C), but widens markedly by 24 h (+8.4 pp). Mechanistically, the isomerization is kinetically controlled within this window: temperature sets the rate, while time determines how far the system has travelled toward the fructose-rich equilibrium, so the two effects reinforce each other. An equivalent analysis on the directly measured optical rotation yields the same dominant factors (time, temperature and their interaction; R^2 = 99.15 %), confirming that the conclusions are robust to the choice of response variable.

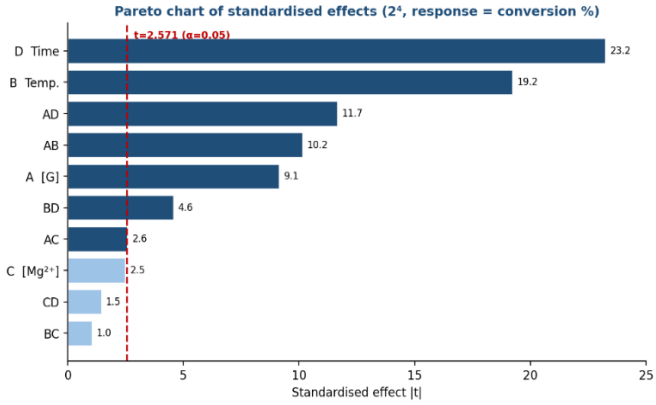

Figure 3. Pareto chart of the standardised effects (2^4, conversion %). Dark bars are significant (|t| ≥ 2.571); light bars are not.

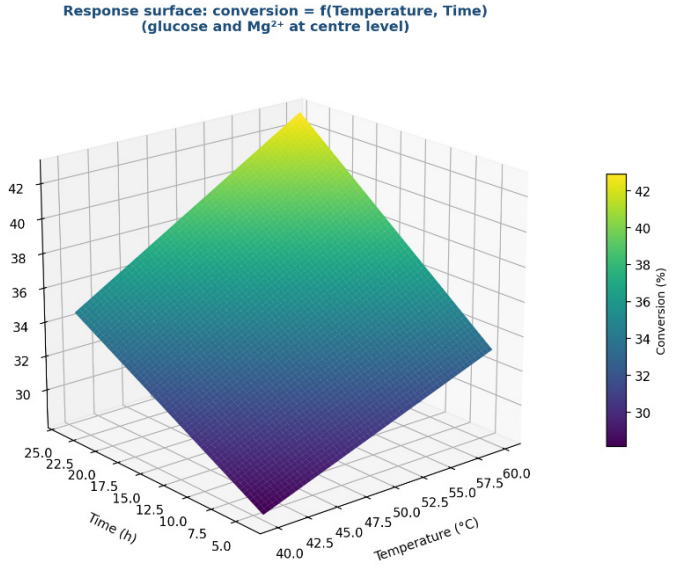

Figure 4. Response surface of conversion as a function of temperature and time (glucose concentration and Mg^{2+} concentration held at their centre levels). The twist of the surface is the visual signature of the temperature × time interaction.

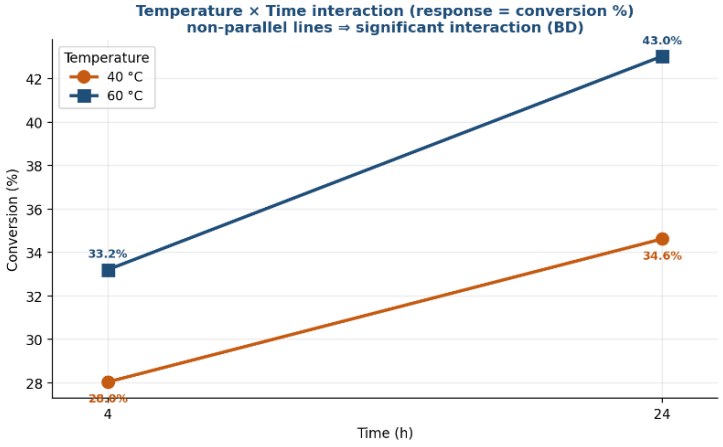

Figure 5. Temperature × time interaction plot (conversion %). Non-parallel lines confirm the significant BD interaction: temperature matters more at long reaction times.

3. Effect of divalent metal cations

Glucose isomerase is a metalloenzyme whose two metal sites (M1, structural; M2, catalytic) require divalent cations. To map the catalyst's activation/inhibition profile, the standard Mg^{2+} activator was compared with Ni^{2+}, Mn^{2+}, Cu^{2+} and Ca^{2+} (0.5 mM salt, 0.6 M glucose, 60 °C, 24 h; Table 5). $MgSO_4 \cdot 7H_2O$ gave the lowest optical rotation and therefore the highest conversion (38.2 %), clearly outperforming all alternatives. Ca^{2+} and Ni^{2+} were the poorest (34.9 % and 34.2 %); the weak performance of Ca^{2+} is consistent with its well-documented role as a competitive inhibitor that displaces Mg^{2+} from the active centre. Ca^{2+} has a significantly larger ionic radius (100 pm) compared to Mg^{2+} (72 pm). When calcium enters the active center, its bulky ionic volume coordinates poorly with the conserved carboxylic residues (such as Asp and Glu) in the M1/M2 pockets. [10, 21] This creates severe steric strain, distorting the precise geometry required for the hydride transfer step and effectively locking the enzyme in an inactive conformation. While Ni^{2+} has a matching ionic radius (69 pm), it forms overly rigid coordination complexes. This locks the carbohydrate intermediate too tightly in the active center, slowing down the product release rate and lowering overall turnover. [22]

These results confirm $MgSO_4 \cdot 7H_2O$ as the superior chemical activator for Sweetzyme IT under the studied conditions.

Table 5. Divalent-cation screening (0.6 M glucose, 60 °C, 24 h). Mg^{2+} (highlighted) is the most effective activator.

Salt	Cation	α (°)	Conversion (%)
$MgSO_4 \cdot 7H_2O$	Mg^{2+}	0.528	38.2
$CuSO_4$	Cu^{2+}	1.685	36.7
$MnCl_2 \cdot 4H_2O$	Mn^{2+}	2.380	35.8
$CaCl_2$	Ca^{2+}	3.102	34.9
$NiCl_2 \cdot 6H_2O$	Ni^{2+}	3.667	34.2

4. Complementary refractometric analysis

In parallel with the polarimetric measurements, every kinetic aliquot was analysed with a digital handheld refractometer, recording three quantities on three scales: °Brix, refractive index (RI) and the instrument's built-in "Fructose % w/w" scale. Measurements were performed for all three temperatures series (40, 50 and 60 °C) on the same 1:10-diluted samples used for polarimetry. The ranges obtained are summarised in Table 6.

Table 6. Range of refractometric readings across all kinetic samples.

Sample series	Total sugar (after 1:10 dilution)	°Brix	Refractive index	Fructose % (w/w)
0.2 M glucose	≈ 0.02 M	0.1 - 0.5	1.3324 - 1.3336	0.1 - 0.5
0.6 M glucose	≈ 0.06 M	0.5 - 0.6	1.3328 - 1.339	0.5 - 0.6
Water baseline	0	0	≈ 1.3330	0

The isomerization process cannot be monitored by refractometric analysis for these particular mixtures. Over each reaction, where polarimetry showed the conversion rising from ~0 % to a maximum of 45.98 % (0.2 M glucose, 60 °C, 24 h), the °Brix, refractive-index and “Fructose % w/w” readings remained essentially flat, changing negligibly between the 0-minute and 24-hour samples. The few elevated points (e.g. RI = 1.339 at 40 °C/0.25 h, °Brix = 0.9 at 60 °C/4 h) are isolated outliers rather than genuine trends. The 1:10 dilution lowered the samples to 0.02–0.06 M (~0.4–1 % w/w sugar), placing the readings at the resolution limit of a handheld refractometer, where they are dominated by noise. What refractometry did capture correctly is the total sugar loading: the 0.6 M series read consistently higher (Brix ≈ 0.5–0.6, RI up to ≈ 1.339) than the 0.2 M series (Brix ≈ 0.2–0.3), reflecting the 3-fold concentration difference.

All quantitative conversion data were obtained by polarimetry [23], which is effective precisely because the two isomers possess large, opposite specific rotations (α_G = +51.22°, α_F = −80.69°), the single property that distinguishes them. Reporting refractometry as a tested but insensitive method strengthens the justification for the polarimetric protocol used throughout this work.

CONCLUSIONS

This work shows that a two-level factorial design provides an efficient, interaction-aware alternative to one-factor-at-a-time optimization of the Sweetzyme IT–catalyzed isomerization of D-glucose to D-fructose. The validated regression models ($R^2 > 0.99$) identify temperature as the dominant operational factor within the 40–60 °C range studied: higher temperature accelerated the approach to equilibrium, the maximum conversion of 45.98 % being reached at 60 °C after 24 h, a value close to the thermodynamic ceiling of the reaction (~45 % fructose) rather than a limit of catalyst efficiency. Operation above ~60 °C is not advisable owing to the well-documented thermal inactivation of the enzyme. Incorporating reaction time as an explicit

factor uncovered a feature inaccessible to single-time-point studies: the rate-controlling variable shifts from substrate concentration at short reaction times to temperature near equilibrium, accompanied by a statistically significant temperature × time interaction. Within the tested range the Mg^{2+} concentration had only a minor effect, indicating that even 0.5 mM nearly saturates the enzyme's metal sites; nevertheless, the comparative cation screening confirmed $MgSO_4 \cdot 7H_2O$ as the most effective activator, clearly outperforming Mn^{2+}, Cu^{2+}, Ni^{2+} and the inhibitory Ca^{2+}. Finally, the dedicated polarimetric calibration offered a rapid, non-destructive route to conversion, whereas refractometry proved intrinsically insensitive to this mass-conserving isomerization.

EXPERIMENTAL SECTION

Materials

D-glucose and D-fructose (Fluka AG), magnesium sulfate heptahydrate (Reactivul Bucuresti), and the commercial immobilized glucose isomerase Sweetzyme IT (Novozymes) were used for the isomerization experiments. Stock solutions of various metal salts, specifically $NiCl_2 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, $CuSO_4$, and $CaCl_2$, were prepared in-house to test cofactor effects.

Equipment

Optical rotation was measured using an ADP220 automatic digital polarimeter (Bellingham & Stanley Ltd) equipped with a 589 nm LED light source. Refractive index and Brix values were determined using a digital handheld refractometer.

Methods

Polarimetric calibration: Standard mixtures with varying molar ratios of glucose to fructose were prepared by diluting 1 M stock solutions in 0.01 M $MgSO_4 \cdot 7H_2O$. The optical rotation of 11 distinct calibration samples was measured at 25 °C using a 100 mm cell length and a wavelength of 589 nm to establish the calibration curves. A 0.01 M $MgSO_4 \cdot 7H_2O$ solution was used as the blank.

Enzymatic isomerization and Factorial design: To optimize the enzymatic conversion of glucose to fructose, a factorial experiment was designed varying three key parameters: temperature (40 and 60 °C), initial glucose concentration (0.2 M and 0.6 M), and Mg^{2+} concentration (0.5 mM

and 1.5 mM). Reactions were performed in 50 mL volumes containing 0.125 g of immobilized Sweetzyme IT. The reaction mixtures were incubated under continuous magnetic stirring at 250 rpm, and the temperature was strictly maintained using a thermostatic water bath. Samples were collected at predetermined intervals: 0, 5, 15, and 30 minutes, followed by 1, 2, 4, and 24 hours. Each collected aliquot was diluted 1:10 with distilled water prior to polarimetric and refractometric measurements to determine the reaction conversion rate.

Effect of metal cations: To investigate the activating or inhibiting effects of different metal ions on the enzyme's specific activity, 50 µL of 0.5 M metal salt solutions (Ni^{2+}, Mn^{2+}, Cu^{2+}, Ca^{2+} and Mg^{2+} as standard) were added to 50 mL of 0.6 M glucose solution. The mixtures, each containing 0.125 g of immobilized enzyme, were incubated at 60 °C. After 24 h the samples were diluted 1:10 and their optical rotation measured against the corresponding 0.01 M salt-solution blanks.

Data analysis: Optical rotations were converted to conversion via the calibration (Eq. 1–3). Factorial effects, ANOVA, regression coefficients and model-quality statistics (R^2, R^2adj, R^2pred) were computed for the two-level designs using Minitab Statistical Software.

ACKNOWLEDGMENTS

This research was supported by the project “Renewable Enzyme Immo bilization - RENZI” funded by European Union – NextGenerationEU and the Romanian Government, under National Recovery and Resilience Plan for Romania, contract no 760241/28.12.2023, cod PNRR-C9-I8-CF 87/31.07.2023 through the Romanian Ministry of Research, Innovation and Digitalization, within Component 9, Investment I8.

REFERENCES

1. K. Parker; M. Salas; V.C. Nwosu, V; *Biotechnol. Mol. Biol. Rev.*, **2010**, *5*, 71-78.
2. J.S. White; *Am. J. Clin. Nutr.*, **2008**, *88*, 1716S-1721S.
3. S.S. Desai; D.B. Gachhi; B.S. Hungund; Glucose Isomerising Enzymes in *Microbial Enzyme Technology in Food Applications,* 1st ed, CRC Press, Boca Raton, Florida, **2016**, 69-84.
4. S. Souzanchi; L. Nazari; K.T.V. Rao; Z.S. Yuan; Z.C. Tan; C.B. Xu; *Catal. Today*, **2019**, *319*, 76-83.

5. A.L.M. Kognou; S. Shrestha; Z. Jiang; C. Xu; F. Sun; W. Qin; *J. Bioresour. Bioprod.,* **2022**, *7*,148-160.
6. H. Li; S. Yang; S. Saravanamurugan; A. Riisager; *ACS Catal.*, **2017**, *7*, 3010-3024.
7. J.M. Carraher; C.N. Fleitman; J.P. Tessonnier; *ACS Catal.*, **2015**, *5*, 3162-3173.
8. S.H. Bhosale; M.B. Rao; V.V. Deshpande; *Microbiol. Rev.*, **1996**, *60*, 280-300.
9. H. Taberman; C.S. Bury; M.J. van der Woerd; E.H. Snell; E.F. Garman; *J. Synchrotron Radiat.*, **2019**, *26*, 931-944.
10. A.Y. Kovalevsky; L. Hanson; S.Z. Fisher; M. Mustyakimov; S.A. Mason; V.T. Forsyth; M.P Blakeley; D.A Keen; T. Wagner, H.L. Carrell, A.K. Katz, J.P. Glusker; P. Langan; *Structure*, **2010**, *18*, 688-699.
11. B. Asbóth; G. Náray-Szabó; *Curr. Protein Pept. Sc.*, **2000**, *1*, 237-254.
12. S.S. Tükel; D. Alagöz; (2008). *Food Chem.*, **2008**, *111*, 658-662.
13. R. DiCosimo; J. McAuliffe; A.J., Poulose; G. Bohlmann; *Chem. Soc. Rev.*, **2013**, *42*, 6437-6474.
14. L. Zittan; P.B. Poulsen; S.H. Hemmingsen; *Starch - Stärke*, **1975**, *27*, 236–241.
15. EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP). *EFSA J.*, **2019**, *17*, e05969.
16. D.C. Montgomery; *Design and Analysis of Experiments*, 10th ed., Wiley, Hoboken, NJ, **2019**.
17. G.E.P. Box; J.S. Hunter; W.G. Hunter; *Statistics for Experimenters: Design, Innovation, and Discovery*, 2nd ed., Wiley-Interscience, Hoboken, NJ, **2005**.
18. R. Leardi; *Anal. Chim. Acta*, **2009**, *652*, 161-172.
19. S.A. Weissman; N.G. Anderson; *Org. Process Res. Dev.,* **2015**, *19*, 1605-1633.
20. C.F. Mandenius; A. Brundin; *Biotechnol. Prog.,* **2008**, *24*, 1191-1203.
21. K.H. Nam; *Appl. Sci.*, **2022**, *12*, 428.
22. R. Bogumil; R. Kappl; J. Hüttermann, *Eur. J. Biochem.*, **1997**, *36*, 12844-12853.
23. S.A Jaywant; H. Singh; K.M. Arif; *Sensors*, **2022**, *22*, 2290.