

Optimization of recyclable citric acid pretreatment for enhanced bioethanol production from heterogeneous starchy food wastes

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Abstract

Background

Sustainable conversion of starchy food waste into bioethanol represents an attractive strategy for reducing food waste while producing renewable biofuels. However, developing environmentally benign pretreatment methods that maximize sugar recovery without generating fermentation inhibitors remains a major challenge. This study optimized a mild citric acid pretreatment for enhancing fermentable sugar release and bioethanol production from diverse starchy food wastes.

Results

A central composite design was employed to optimize citric acid concentration (1–5%, w/v), pretreatment temperature (90–130°C), and reaction time (20–60 min). The quadratic model was highly significant ($R^2 = 0.969$), with pretreatment temperature exerting the greatest influence on reducing sugar production, followed by citric acid concentration. The optimum conditions (3% citric acid, 130°C, and 40 min) yielded $30.99 \pm 1.80 \text{ g L}^{-1}$ reducing sugars from kitchen food waste. An indigenous yeast isolate was identified as *Saccharomyces cerevisiae* (ITS rRNA; GenBank accession no. PZ683636) and applied in simultaneous saccharification and fermentation. Under the optimized conditions, citric acid pretreatment combined with amylase supplementation produced $54.04 \pm 1.37 \text{ g L}^{-1}$ ethanol within 24 h. Although sulfuric acid pretreatment generated higher reducing sugar concentrations, citric acid pretreatment achieved superior ethanol production because of markedly lower hydroxymethylfurfural formation (2.66 ± 0.03 versus $5.82 \pm 0.11 \text{ g L}^{-1}$). Application of the optimized process to different starchy food wastes generated 28.58–68.49 g L^{-1} reducing sugars and 45.28–66.74 g L^{-1} ethanol, with damaged sorghum grains and rotten potato biomass producing the highest ethanol concentrations. Moreover, $91.94 \pm 0.02\%$ of the applied citric acid was recovered after pretreatment.

Conclusions

The optimized citric acid pretreatment provides an efficient and environmentally sustainable alternative to conventional mineral acid pretreatments by enhancing ethanol production while minimizing inhibitor formation. Combined with high citric acid recovery, this strategy offers a promising approach for the circular biorefinery valorization of starchy food waste into bioethanol.

1. Introduction

The global energy dependence on petroleum-derived fuels has resulted in significant and cumulative environmental problems, such as the intensification of the greenhouse effect, global warming, and the ensuing climate change that pose significant risks to ecosystems and human societies [1]. As an alternative, biofuels have been gaining significant attention owing to their low carbon impact, thus enabling them to mitigate the environmental footprint of fossil fuel consumption [2]. Among these, bioethanol is a promising

renewable substitute for gasoline, offering direct compatibility with existing transportation infrastructure, a high-octane number, and the potential to reduce carbon emissions [3].

Within a circular economy framework, waste feedstocks rich in sugars or starch are ideal for sustainable ethanol production [4]. However, not all feedstocks are suitable; limitations include low biocatalyst tolerance, inhibitory compounds, or poor hydrolysis efficiency of carbohydrates, particularly in lignin-rich materials [5]. Production of first-generation bioethanol generally employs edible biomass sources such as corn and sugarcane; however, the use of food-based materials poses some significant challenges related to food security, competition between crops and water usage. Consequently, a growing interest has been paid in using low-cost residue, especially food waste to produce bioethanol [6]. Starchy food residues, encompassing discarded bread, rice, pasta, damaged cereal grains, spoiled tubers, and processing effluents, are particularly attractive owing to their high carbohydrate content, widespread availability, and the negative costs associated with waste disposal [7–11]. Because these materials were originally intended for human consumption, they are generally free of toxic compounds and align closely with the principles of the circular economy, wherein wastes are valorized into energy and value-added products.

In starchy food wastes, starch occurs in the form of granules with semi-crystalline, lamellar structure stabilized by strong intermolecular hydrogen bonds and thus is resistant to both enzymatic hydrolysis and microbial fermentation [12]. Accordingly, the effectiveness of enzymes such as α -amylase and glucoamylase to depolymerize starch into glucose is critically limited by substrate accessibility, particularly in complex food waste matrices where starch is embedded within protein, lipid, and fiber networks [13]. Consequently, thermochemical pretreatment is required to destabilize starch granules and make them more susceptible to enzyme action and improve ethanol yields [1].

Conventional pretreatment methods for food wastes involve the use of mineral acids such as sulfuric acid and hydrochloric acid owing to their high hydrolyzing capabilities and high sugar yields [6, 7]. However, mineral acids are highly corrosive, toxic, and lead to the formation of inhibitors such as hydroxymethylfurfural (HMF) and furfural, thus interfering with the fermentation process and downstream processing costs [14]. In addition, pretreatment using mineral acids demands the use of special corrosion-resistant reactors, thereby limiting process sustainability and economic feasibility [14, 15]. On the other hand, organic acids have attracted more interest as an environmentally friendly alternative since they are relatively less toxic, help in improving biomass digestibility, and reduce inhibitor formation [16]. Organic acid pretreatment has also been associated with improved enzymatic hydrolysis efficiency and enhanced downstream bioconversion performance. Although several studies have explored organic acid pretreatment for lignocellulosic biomass and agricultural residues, limited attention has been given for the utilization of organic acids for the pretreatment of heterogeneous starchy food wastes for improving bioethanol production. The pretreatment of sugarcane bagasse using citric acid (6.0 wt%, 102.4 min) have been reported to reduce lignin and crystallinity of biomass, leading to higher total reducing sugars yields after enzymatic hydrolysis [17]. Citric acid pretreatment (6%, 121°C, 60 min) outperformed sulfuric acid pretreatment (3%, 121°C, 60 min) during enzymatic hydrolysis of coffee husk, yielding higher glucose (3.88%) and reducing sugar (17.02%) production, while offering a less toxic and more environmentally friendly alternative [18]. In another study, Kitiborwornkul et al. (2023) reported that citric acid pretreatment yielded the highest ethanol conversion from coffee shell

(83 mg g⁻¹ biomass), outperforming H₂SO₄ pretreatment despite similar reducing sugar release, while also removing hemicellulose and offering lower toxicity and corrosiveness than inorganic acids [19].

The present study investigated the application of a mild and cost-effective citric acid pretreatment to enhance bioethanol production from various starchy food wastes. Initially, the pretreatment process was optimized by estimating the effects of citric acid concentration, reaction temperature, and time to determine the optimal pretreatment conditions. Subsequently, simultaneous hydrolysis and ethanol fermentation were evaluated using different starchy food waste substrates. The findings provide a novel and sustainable tactic for the pretreatment of starchy food wastes, highlighting the potential of citric acid as an environmentally friendly alternative pretreatment strategy for improving bioethanol production.

2. Materials and methods

2.1. Food waste collection and analyses

Samples of starchy food waste (SFW) were collected from multiple sources in Assiut Governorate, Egypt, including households, restaurants, and other food service establishments. Kitchen SFW consisted mainly of leftover bread, rice, and pasta (Fig. 1a). Damaged cereal grains, including wheat, corn, and sorghum, were also utilized. The damage includes broken, cracked, and infected grains with fungi/insects (Fig. 1b-e). In addition, infected/rotted potato tubers and sweet potato roots were included as starchy residues (Fig. 1f, g). A potato-processing wastewater stream generated during the washing stage at a potato chips factory was also collected and used as SFW feedstock (Fig. 1h). The collected samples were dried at 50–55°C, followed by grinding in a laboratory blender to obtain a homogenous powder with a particle size less than 0.5 mm. The physicochemical characteristics of the samples (Table 1), including moisture content, pH, total solids, ash, protein, lipids, starch, and metal content were analyzed according to standard methods [20].

Table 1
Physicochemical characteristics of the collected starchy food waste.

Parameters	Kitchen wasted food	Damaged maize grains	Damaged wheat grains	Damaged sorghum grains	Damaged sweet potato biomass	Damaged potato biomass	Potato waste stream
Humidity (%)	57.60	1.60	5.00	4.50	77.15	78.34	88.00
Total solids (%)	42.40	98.40	95.00	95.50	22.85	21.66	12.00
PH	7.20	6.33	6.97	7.00	7.11	9.90	6.60
Starch (%)	38.28	45.92	28.36	39.29	40.14	51.31	56.99
Ash (%)	4.85	2.00	4.00	3.96	4.72	8.91	0.99
Protein (%)	14.72	13.17	13.96	12.87	4.86	15.27	3.54
Lipids (mg g ⁻¹)	7.65	10.84	11.09	10.26	9.09	11.84	11.64
Amino acid (mg g ⁻¹)	74.91	125.84	125.76	159.65	104.07	79.10	96.86
Reducing sugars (mg g ⁻¹)	112.49	10.29	247.51	7.65	0.11	18.21	6.90
Cd (mg kg ⁻¹)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cr (mg kg ⁻¹)	1.18	1.49	1.05	1.48	1.27	2.09	1.71
Cu (mg kg ⁻¹)	1.08	1.53	0.75	1.69	4.32	1.82	1.17
Fe (mg kg ⁻¹)	135.15	135.76	136.96	125.33	149.71	198.73	120.71
Pb (mg kg ⁻¹)	0.18	0.26	0.49	0.18	0.22	0.14	0.27
Zn (mg kg ⁻¹)	3.23	7.12	7.67	8.50	3.26	5.10	1.75

2.2. Isolation of yeast and molecular identification

Yeast was isolated from naturally fermented food wastes collected from households and restaurants in Assiut Governorate, Egypt. Approximately 10 g of the fermented food waste was aseptically suspended in 90 mL of sterile physiological saline solution (0.85% NaCl) and homogenized by vigorous shaking. Serial decimal

dilutions were prepared, and 100 μL aliquots of appropriate dilutions were spread onto Yeast Malt Extract (YME) agar plates. The YME medium consisted of (g L^{-1}): glucose, 10.0; peptone, 5.0; yeast extract, 3.0; malt extract, 3.0; and agar, 20.0, with the pH adjusted to 6.5 before sterilization. The inoculated plates were incubated at 30°C for 48–72 h. Colonies exhibiting distinct yeast morphology were selected and repeatedly streaked onto fresh YME agar plates until pure cultures were obtained.

Genomic DNA was extracted from the yeast isolate and sent to MacroGen Clinical Laboratory (South Korea) for sequencing the internal transcribed spacer (ITS) region of the rRNA gene using the universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). PCR amplification was performed under standard cycling conditions, and the amplified products were purified and sequenced bidirectionally using the same primer pair. The resulting sequences were assembled and compared with reference sequences in the NCBI GenBank database using the BLAST algorithm for preliminary identification. Multiple sequence alignment was performed using ClustalW, and a phylogenetic tree was constructed to confirm the taxonomic position of the isolate.

2.3. Optimization of acidic pretreatment of SFW using citric acid

Acidic hydrolysis of kitchen leftover SFW was carried out using 1–5% (w/v) citric acid for 20–60 min in an autoclave at a controlled temperature range of 90–130°C. The influence of acid concentration, reaction temperature, and hydrolysis time on the release of reducing sugars was assessed using a central composite design (CCD). Each factor was tested at five levels, which were coded as $-\alpha$ (axial point), -1 (low), 0 (center), 1 (high), and α (axial point). The CCD included 17 experimental runs with 3 replicates at the center point, as summarized in Table 2. The CCD was based on the following general equation:

Table 2

Effect of different concentrations of citric acid at different temperatures and periods in terms of actual and coded levels on the production of reducing sugars from starchy food waste.

#	Citric acid (% w/v)	Temperature (°C)	Time (min)	Reducing sugars (g L ⁻¹)	Reducing sugars (mg g ⁻¹)
1	2 (-1)	100 (-1)	30 (-1)	4.95	24.77
2	2 (-1)	100 (-1)	50 (1)	5.50	27.51
3	2 (-1)	120 (1)	30 (-1)	14.44	72.22
4	2 (-1)	120 (1)	50 (1)	15.79	78.95
5	4 (1)	100 (-1)	30 (-1)	5.09	25.46
6	4 (1)	100 (-1)	50 (1)	6.57	32.84
7	4 (1)	120 (1)	30 (-1)	24.28	121.39
8	4 (1)	120 (1)	50 (1)	23.21	116.04
9	1 (-α)	110 (0)	40 (0)	5.63	28.15
10	5 (α)	110 (0)	40 (0)	11.37	56.83
11	3 (0)	90 (-α)	40 (0)	4.43	22.16
12	3 (0)	130 (α)	40 (0)	30.58	152.92
13	3 (0)	110 (0)	20 (-α)	7.39	36.93
14	3 (0)	110 (0)	60 (α)	14.68	73.40
15*	3 (0)	110 (0)	40 (0)	8.69	43.45
* Mean value of 6 replicates at the center point					

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j$$

1

where Y is the predicted reducing sugars' concentration, β_0 , β_i , β_{ii} and β_{ij} are the model intercept, linear, quadratic, and interaction coefficients, respectively. While X_i and X_j are independent variables.

For each treatment, food waste was suspended in citric acid solution at solid: liquid ratio of 20% w/v. Following thermo-acidic hydrolysis, the mixture was centrifuged at 5000 rpm to separate the solid residue from the liquid hydrolysate.

2.4. Determination of total reducing sugars and HMF in hydrolysates

Reducing sugars in the acidic hydrolysate was measured by 3,5-dinitrosalicylic acid method (Miller, 1959). The reaction contained 1 mL sample and 1 mL reagent, and the mixture was heated at 90°C for 10 min. The developed color was measured at 540 nm using UV-Vis spectrophotometer against glucose as a standard.

The concentration of 5-hydroxymethylfurfural (HMF) in the hydrolysates was determined spectrophotometrically as described previously [21], with slight modifications. Samples were centrifuged to remove suspended solids, and absorbance was measured at 284 and 336 nm using a UV-Visible spectrophotometer. The absorbance at 336 nm was used to correct for background interference, and the HMF concentration was calculated from the difference between the two absorbance values. Quantification was performed using a calibration curve prepared with standard HMF solutions of known concentrations.

2.5. Recovery of citric acid

The citric-acid hydrolysate was neutralized using $\text{Ca}(\text{OH})_2$ powder, which facilitated the precipitation of citric acid as insoluble calcium citrate. The precipitation was completed at 50°C in 20 min. The precipitate was subsequently removed by filtration. For the quantification of recovered citric acid, a 0.1 g of dried calcium citrate precipitate was dissolved in 0.1 M HCl and the concentration of released citric acid was determined spectrophotometrically [22] using pyridine-acetic anhydride method [23].

2.6. Simultaneous saccharification and fermentation

2.6.1. Fermentation conditions

The citric acid-free hydrolysate was recombined with the solid residue, and the pH was carefully adjusted to 5.5–6.0 before sterilization by autoclaving and subsequent fermentation for bioethanol production.

The fermentation process was performed in 100 mL serum vials sealed with rubber stoppers and fitted with sterilized needles for CO_2 release. Amylase enzyme was added to the fermentation medium at a dosage of 10 U g^{-1} initial food waste to facilitate the hydrolysis of residual starch during fermentation. The fermentation medium was inoculated with 10% (v/v) of yeast suspension (1×10^6 cells mL^{-1}), and ethanol production was proceeded at 37°C and 150 rpm for 72 h.

2.6.2. Ethanol quantification

The bioethanol concentration in the fermentation broth was estimated using potassium dichromate method [24] using absolute ethanol as standard. The accuracy and reliability of the dichromate assay were evaluated by comparison with high-performance liquid chromatography (HPLC) analysis performed at the Analytical Chemistry Unit, Faculty of Science, Assiut University.

2.7. Statistical analyses

All the experiments were performed in triplicates. Central Composite Design (CCD) analyses were performed using Chemoface software version 1.75 [25]. Pareto charts and response surface plots were also generated using the same software package to evaluate the effects and interactions of the studied variables.

3. Results

3.1. Physicochemical characteristics of starchy food wastes

The physicochemical characteristics of the collected starchy food wastes are presented in Table 1. Considerable variation was observed among the different substrates. Potato waste stream exhibited the highest starch content (56.99%), followed by damaged potato biomass (51.31%) and damaged maize grains (45.92%), whereas damaged wheat grains contained the highest reducing sugar content (247.51 mg g⁻¹). Moisture content ranged from 1.60% in damaged maize grains to 88.00% in potato waste stream, with the corresponding total solids showing an inverse trend. Protein content varied between 3.54 and 15.27%, while lipid concentrations ranged from 7.65 to 11.84 mg g⁻¹. Damaged sorghum grains contained the highest amino acid content (159.65 mg g⁻¹). Analysis of heavy metals revealed that cadmium was not detected in any sample, whereas chromium, copper, iron, lead, and zinc were present at relatively low concentrations. Among these elements, iron was the predominant metal, with the highest concentration recorded in damaged potato biomass (198.73 mg kg⁻¹).

3.2. Molecular identification of the yeast isolate

The ITS region of the rRNA gene was successfully amplified from the yeast isolate using the universal primers ITS1 and ITS4, yielding a high-quality sequence of 865 bp. BLAST analysis of the obtained ITS sequence against the NCBI GenBank database identified the isolate as *Saccharomyces cerevisiae* (accession number PZ683636). The sequence exhibited 99.28–100% identity and 88–97% query coverage with several *S. cerevisiae* strains deposited in GenBank, including the type strain *S. cerevisiae* ATCC 18824 (GenBank accession no. AY046146). Phylogenetic analysis based on ITS sequences further confirmed the taxonomic placement of the isolate within the *S. cerevisiae* clade, clearly separating it from the outgroup species, *Candida albicans* (Fig. 2). These results unequivocally confirmed that the isolated yeast strain belonged to the species *Saccharomyces cerevisiae* (isolate STDF-068).

3.3. Optimization of citric acid pretreatment of starchy food wastes

The impacts of citric acid treatment at different temperatures and periods on the production of reducing sugars from kitchen-derived SFW were listed in Table 2. The experimental relationship between the response and the investigated factors was developed based on the CCD results (Table 3). Analysis of variance (ANOVA) results showed high F-values, indicating that the developed model was statistically significant ($p \leq 0.0001$). The high determination coefficient values ($R^2 = 0.969$) and adjusted determination coefficient values (adjusted $R^2 = 0.958$) for the model further suggest the high effectiveness and adequacy of the model. The regression equation (Eq. (1)) was:

Table 3
Coefficients and significance tests of the variables obtained for the hydrolysis of starchy food waste with citric acid.

Constant (b_0)	Coefficients (b)	Error	t-value	p-value
	261.68	26.90	9.727	< 0.0001
Citric acid concentration (X_1)	-19.43	3.28	-5.928	< 0.0001
Temperature (X_2)	-4.79	0.40	-12.012	< 0.0001
Time (X_3)	-0.10	0.33	-0.309	0.76
$X_1 * X_2$	0.20	0.03	7.553	< 0.0001
$X_1 * X_3$	-0.02	0.03	-0.702	0.49
$X_2 * X_3$	-0.002	0.003	-0.824	0.42
X_1^2	-0.003	0.17	-0.016	0.99
X_2^2	0.02	0.002	13.181	< 0.0001
X_3^2	0.006	0.002	3.698	0.0015

$$\text{Reducing sugars} = 261.68 - 19.43X_1 - 4.79X_2 - 0.1X_3 + 0.2X_1X_2 - 0.02X_1X_3 - 0.002X_2X_3 - 0.003(X_1)^2 + 0.02(X_2)^2 + 0.006(X_3)^2$$

where X_1 , X_2 , and X_3 are the coded levels for the citric acid concentration, temperature and time, respectively.

The results indicated the production of $4.95\text{--}30.85 \text{ g L}^{-1}$ under different pretreatment conditions (Table 2). The effect of the investigated variables and their interactions on the production of reducing sugars from starchy food waste were statistically estimated and the results were presented in Pareto chart (Fig. 3). The objective of the Pareto chart is to summarize the significance of the linear, quadratic, and interaction effects of citric acid concentration (X_1), pretreatment temperature (X_2), and pretreatment time (X_3) on fermentable sugar production from starchy food waste. The dotted line is related to the value of p ; the variables or interactions that exceed this line are those that represent significant effects on the response variable. Furthermore, three 3D response surface graphs were constructed, showing the effects of the variables on the production of reducing sugars (Fig. 4). The results depicted in Fig. 3 The Pareto chart revealed that pretreatment temperature was the most influential factor affecting fermentable sugar production. Although the linear effect of temperature was negative, the quadratic effect was positive and exhibited the largest standardized effect, indicating a pronounced curvature in the response surface. Consequently, reducing sugar production initially decreased over part of the experimental range but increased markedly at higher temperatures, as illustrated by the response surface plot (Fig. 3). This behavior indicates that citric acid pretreatment was governed primarily by the quadratic temperature effect rather than by the linear component alone (Table 3, Fig. 3). On the other hand, at relatively low temperatures, increasing citric acid alone may not

sufficiently hydrolyze starch and may even contribute to incomplete gelatinization. Therefore, citric acid concentration exhibited a significant negative linear coefficient (Table 3, Fig. 3). This result indicated that the local linear trend of citric acid around the design center was negative, whereas the overall response depended on the combined influence of all model terms. Consequently, when combined with elevated temperatures, citric acid effectively catalyzes starch hydrolysis, leading to a synergistic increase in reducing sugar production. This synergism is reflected by the significant positive interaction between citric acid concentration and temperature (X_1X_2) (Fig. 3, 4). Conversely, the interactions acid $\times$ time and temperature $\times$ time were not statistically significant ($p > 0.05$), suggesting that time did not strongly influence the combined effects of acid or temperature. The quadratic analysis of time is significant, indicating optimal operating ranges rather than simple linear effects (Table 3). In contrast, acid concentration exhibited non-significant quadratic behavior, indicating that its effects remained largely linear within the tested range.

3.4. Ethanol production

Figure 5 depicts ethanol production (g L^{-1}) from wasted food under various pretreatment and enzymatic treatment conditions. In the absence of acidic pretreatment and amylase addition, ethanol production was negligible ($< 1 \text{ g L}^{-1}$). Incorporating amylase during yeast fermentation without acidic pretreatment exhibited a significant enhancement of ethanol yields to $11.53 \pm 1.37 \text{ g L}^{-1}$ within 48 h. Similarly, citric acid pretreatment without amylase incorporation during fermentation led to a substantial increase in ethanol production, reaching $15.33 \pm 1.27 \text{ g L}^{-1}$ within 24 h, with a marked reduction at longer fermentation periods (Fig. 5). Conversely, the sulfuric acid pretreatment without amylase incorporation induced significantly higher ethanol yields, reaching $31.13 \pm 0.39 \text{ g L}^{-1}$ within 24 h, and increased to $43.41 \pm 1.95 \text{ g L}^{-1}$ at 96 h. On the other hand, the combined treatment (citric acid pretreatment and amylase incorporation during fermentation) produced significantly highest ethanol yields of $54.04 \pm 1.37 \text{ g L}^{-1}$ after 24 h. In contrast, the combined sulfuric acid pretreatment in the presence of amylase produced $44.86 \pm 1.85 \text{ g/L}$ after 24 h, which was increased to $54.11 \pm 0.49 \text{ g L}^{-1}$ after 72 h.

The comparative effect of organic acid (citric acid, 3% w/v) and mineral acid (H_2SO_4 , 1% w/v) pretreatment under autoclave conditions (130°C , 40 min) on the conversion of starchy food waste is presented in Table 4. H_2SO_4 produced markedly higher reducing sugar release ($57.21 \pm 0.66 \text{ g L}^{-1}$) compared to citric acid pretreatment ($30.99 \pm 1.80 \text{ g L}^{-1}$). However, H_2SO_4 pretreatment produced markedly higher concentrations of hydroxymethylfurfural (HMF), reaching $5.82 \pm 0.11 \text{ g L}^{-1}$, compared to $2.66 \pm 0.03 \text{ g L}^{-1}$ in citric acid-treated samples. Despite the lower sugar release, citric acid pretreatment resulted in superior fermentation performance. Ethanol yield after 24 h reached $54.04 \pm 1.37 \text{ g L}^{-1}$, compared to $44.86 \pm 1.85 \text{ g L}^{-1}$ for H_2SO_4 pretreatment. Similarly, ethanol conversion and productivity were higher in citric acid-treated samples ($270.20 \pm 6.83 \text{ g kg}^{-1}$ biomass, $2.25 \pm 0.06 \text{ g L}^{-1} \text{ h}^{-1}$) than in H_2SO_4 -treated samples ($224.32 \pm 9.27 \text{ g kg}^{-1}$ biomass, $1.87 \pm 0.08 \text{ g L}^{-1} \text{ h}^{-1}$).

Table 4

Comparison between organic acid pretreatment (citric acid 3% w/v) and mineral acid pretreatment (H_2SO_4 1% w/v) at 130°C for 40 min on the production of reducing sugar release, hydroxymethyl furfural formation, and bioethanol production from starchy food waste.

Pretreatments	Reducing sugars (g L^{-1})	Reducing sugars (mg g^{-1})	HMF (g L^{-1})	Ethanol yield (24 h) (g L^{-1})	Ethanol conversion (24 h) (g kg^{-1} biomass)	Ethanol productivity (24 h) ($\text{g L}^{-1} \text{h}^{-1}$)
Citric acid	30.99 ± 1.80	154.94 ± 8.98	2.66 ± 0.03	54.04 ± 1.37	270.20 ± 6.83	2.25 ± 0.06
H_2SO_4	57.21 ± 0.66	286.03 ± 3.30	5.82 ± 0.11	44.86 ± 1.85	224.32 ± 9.27	1.87 ± 0.08
All the comparisons between citric acid and H_2SO_4 were statistically significant using analysis of variance (ANOVA) at $p < 0.05$.						

The optimum citric acid pretreatment conditions were applied into different wastes, resulting in 28.58–68.49 g L^{-1} of reducing sugars (Fig. 6a). Bioethanol production from these substrates, following citric acid pretreatment and amylase incorporation during fermentation, is depicted in Fig. 6b. Fermentation of damaged cereal grains led to rapid ethanol production, with substantial yields achieved within 24 h. In contrast, higher ethanol yields from rotten potato biomass, sweet potato biomass, and potato waste stream were obtained after extended fermentation periods of 48–72 h. Within 24 h, damaged sorghum grains exhibited the highest ethanol yield ($64.39 \pm 5.00 \text{ g L}^{-1}$), followed by damaged wheat grains ($52.18 \pm 2.63 \text{ g L}^{-1}$) and damaged maize grains ($50.18 \pm 3.71 \text{ g L}^{-1}$) (Fig. 6b). Conversely, the maximum ethanol yields for tuber-based wastes were achieved after 72 h, reaching $66.74 \pm 6.64 \text{ g L}^{-1}$ from rotten potato biomass, $52.38 \pm 5.00 \text{ g L}^{-1}$ from rotten sweet potato biomass, and $45.28 \pm 9.46 \text{ g L}^{-1}$ from waste potato stream (Fig. 6b). The ethanol conversion yields in relation to the solid biomass loadings were fluctuated between 122.54 g kg^{-1} biomass and 333.69 g kg^{-1} biomass (Fig. 6c).

3.5. Citric acid recovery

A recovery efficiency of $91.94 \pm 0.02\%$ of citric acid from the food waste hydrolysate was achieved via precipitation with $\text{Ca}(\text{OH})_2$, followed by conversion of the resulting calcium citrate into citric acid using H_2SO_4 . This result implied that approximately 27.58 g of citric acid could be recovered from 1.0 L of hydrolysate, requiring only about 2.42 g of fresh citric acid as make-up for the subsequent pretreatment cycle.

4. Discussion

The present study demonstrated the effectiveness of citric acid in the pretreatment of different starchy food waste. Among the investigated parameters, pretreatment temperature was identified as the most influential factor affecting fermentable sugar production from kitchen starchy food waste, followed by citric acid concentration. The response surface showed that fermentable sugar production increased markedly at elevated temperatures, particularly above approximately 110°C. This behavior is expected because higher

temperatures promote starch gelatinization by disrupting the crystalline structure of starch granules, thereby increasing their accessibility to acid-catalyzed hydrolysis. Elevated temperatures also accelerate the cleavage of α -1,4- and α -1,6-glycosidic bonds, enhancing the conversion of starch into soluble oligosaccharides and reducing sugars. At lower temperatures, citric acid alone produced limited starch hydrolysis because the starch granules remained relatively intact. In contrast, higher temperatures promoted starch gelatinization and increased the accessibility of glycosidic bonds, allowing citric acid to catalyze starch depolymerization more effectively. Consequently, the combined application of elevated temperature and citric acid enhanced fermentable sugar production more than either factor alone, demonstrating a synergistic effect. Similar synergistic interactions between acid concentration and temperature have been reported for dilute-acid hydrolysis of biomass, where elevated temperatures accelerate reaction kinetics while the acid catalyzes polysaccharide hydrolysis [26, 27].

Optimal conditions (3% w/v citric acid, 130°C, and 40 min) yielded 154.94 mg g⁻¹ of fermentable sugars. Supporting this mechanism, Kitiborwornkul et al. observed a 1.62-fold increase in reducing sugar yield following citric acid pretreatment of coffee shell, along with enhanced cellulose accessibility [19]. However, harsher conditions of 11.7% (w/v) citric acid, 137.4°C, and 240 min were required to release 271.6 mg g⁻¹ of reducing sugars from coffee shell [19]. Likewise, Dharmalingam et al. showed that acetic acid effectively removed lignin and hemicellulose from mixed vegetable waste, producing high reducing sugar yields (up to 314 mg g⁻¹ biomass) at 68.32 min, 140°C, and acid concentration of 10% (w/v) [28]. Citric acid facilitates starch hydrolysis by weakening the internal hydrogen-bond network within starch granules, thereby increasing chain mobility, water absorption, swelling, solubility, and susceptibility to enzymatic attack [12].

The results indicated that both pretreatment and enzymatic hydrolysis are crucial for efficient ethanol production from starchy food wastes. Without acid pretreatment or amylase supplementation, ethanol production was negligible (< 1 g L⁻¹), confirming that native starch was largely inaccessible to *Saccharomyces cerevisiae*, as previously reported [13, 29]. The incorporation of amylase alone increased ethanol yield to 11.53 ± 1.37 g L⁻¹, highlighting the role of enzymatic starch hydrolysis. Citric acid pretreatment significantly enhanced ethanol production (15.33 ± 1.27 g L⁻¹ at 24 h), likely due to improved starch solubilization with limited inhibitor formation [30], although yields declined at longer fermentation times. In contrast, sulfuric acid pretreatment produced substantially higher ethanol concentrations, reaching 43.41 ± 1.95 g L⁻¹ at 96 h, reflecting its strong hydrolytic capacity, albeit with slower fermentation kinetics likely due to the formation of inhibitory compounds as reported earlier [31]. The highest ethanol yield was achieved when citric acid pretreatment was combined with amylase, reaching 54.04 ± 1.37 g L⁻¹ at 24 h. This indicated a pronounced synergistic effect that maximized sugar availability while minimizing inhibitory impacts [32]. Although sulfuric acid with amylase reached a comparable final yield, its slower kinetics suggest that mild organic acid pretreatment is more advantageous for rapid and efficient bioethanol production from food waste. Furthermore, mineral acids are also hazardous, poisonous, and corrosive, necessitating corrosion-resistant reactors, which drive up the cost of the pretreatment process. Citric acid avoids these infrastructure requirements and safety concerns while producing cleaner fermentation profiles with fewer inhibitory by-products. Moreover, from economic and sustainability perspectives, citric acid can be effectively recycled via precipitation with calcium hydroxide, followed by regeneration using sulfuric acid, and gypsum was formed as a byproduct. This recovery route is a well-established method for the industrial recovery of citric acid from

fermented broth [33]. The high recovery efficiency ($91.94 \pm 0.02\%$) indicates that only about 2.42 g of fresh citric acid is required per liter of hydrolysate, enabling a nearly closed-loop system. This approach of waste recycling aligns with the concept of biorefinery and circular bioeconomy, emphasizing energy, nutrient, and material recovery.

The enhanced efficiency of citric acid treatment led to a significant increase in fermentable sugars extracted from a wide variety of starchy food wastes, reaching up to $28.58\text{--}68.49 \text{ g L}^{-1}$ of reducing sugars. The differences among substrates may be attributed to variations in starch composition, starch granule structure, and the presence of non-starch components that influence hydrolysis efficiency. Damaged cereal grains such as sorghum, wheat, and maize yielded relatively high amounts of fermentable sugars, which were efficiently fermented to ethanol within the first 24 hours of SSF. Cereal grains generally contain highly digestible starch with lower moisture content and a more favorable amylose/amylopectin ratio, facilitating rapid enzymatic saccharification and subsequent yeast fermentation [34, 35]. The maximum ethanol yield in the first 24 h was attained from damaged sorghum grains ($64.39 \pm 5.00 \text{ g L}^{-1}$), followed by damaged wheat grains ($52.18 \pm 2.63 \text{ g L}^{-1}$) and maize grains ($50.18 \pm 3.71 \text{ g L}^{-1}$). Sorghum is considered one of the most efficient sources of bioethanol, due to its high starch content and carbohydrate composition capable of being easily converted to fermentable sugars [36]. In contrast, tuber-derived wastes required longer fermentation periods to achieve maximum ethanol yields. Rotten potato biomass produced the highest ethanol concentration after 72 h ($66.74 \pm 6.64 \text{ g L}^{-1}$), followed by rotten sweet potato biomass ($52.38 \pm 5.00 \text{ g L}^{-1}$) and potato waste stream ($45.28 \pm 9.46 \text{ g L}^{-1}$). Potatoes and sweet potatoes contain starch granules with larger particle sizes and distinct crystalline structures that often require more extensive hydrolysis before complete fermentation can occur [37]. Similar delayed fermentation kinetics have been reported for potato-based bioethanol production systems, where prolonged fermentation periods improved sugar utilization and ethanol yields [11].

As summarized in Table 5, the ethanol concentrations obtained in this work ($50.18\text{--}64.39 \text{ g L}^{-1}$) are among the highest reported for comparable waste feedstocks, and they highlight the efficacy of combining a mild organic-acid pretreatment with SSF at a high solid loading of 20% (w/v). For kitchen SFW, the ethanol concentration obtained in the present work (54.04 g L^{-1}) was substantially higher than those reported by Hashem et al., Al-Malki et al., and Hafid et al., who achieved only $10.92\text{--}15.62 \text{ g L}^{-1}$ ethanol despite employing mineral acid hydrolysis followed by fermentation [6–8]. The superior performance observed here may be attributed to the use of higher substrate loading (20% w/v), the efficient disruption of starch-rich matrices by citric acid pretreatment at elevated temperature, and the integration of enzymatic saccharification with fermentation in a single SSF process. SSF minimizes glucose accumulation and product inhibition of amylolytic enzymes while simultaneously reducing contamination risks, thereby enhancing ethanol productivity. Although Ntaikou et al. reported higher ethanol yields ($42.19\text{--}83.30 \text{ g L}^{-1}$), their process relied on combined acid and enzymatic hydrolysis and extended fermentation periods up to 72 h [1]. Likewise, Kiran and Liu achieved 58 g L^{-1} ethanol using a concentrated glucose-rich hydrolysate generated by a fungal enzyme cocktail [38]. Therefore, the ethanol yield obtained in this study is comparable to the best-performing literature reports while employing a relatively simple and environmentally benign pretreatment strategy. For damaged maize grains, the ethanol yield of 50.18 g L^{-1} exceeded the 42.4 g L^{-1} reported by Gawande and Patil [9]. It was also markedly higher than the values reported by Paika et al., who obtained only 2.9–3.6% (v/v) ethanol following liquefaction and saccharification with crude enzymes [10]. The ethanol concentration

obtained from damaged wheat grains (52.18 g L^{-1}) was considerably higher than the 4.4% (v/v) reported by Suresh et al., highlighting the advantages of the current pretreatment-fermentation approach [39]. However, it remained lower than the 69 g L^{-1} reported by Vidmantiene et al., who utilized a more intensive hydrolysis strategy involving thermal treatment, liquefaction, and saccharification with multiple enzyme preparations [40]. The difference suggests that although the simplified citric acid-based process may not maximize starch conversion to the same extent as multi-enzyme systems, it offers a practical balance between process simplicity, cost reduction, and ethanol productivity. Similarly, the ethanol yields obtained from waste potato and sweet potato biomasses also compared favorably with previous reports [11, 41–43].

Table 5

Comparison of bioethanol production from starch-rich wastes under different pretreatment and fermentation strategies reported in the literature and the present study.

Biomass	Pretreatment	Substrate loading	Microorganism	Fermentation conditions	Ethanol yield	Reference
Kitchen SFW	Acidic hydrolysis: 1M HCl, pH 1, 121°C autoclaving for 30 min	25 (% w/v)	<i>S. cerevisiae</i>	SHF: pH 5, 35°C, 5% inoculum, 4 days	11.57 g L ⁻¹	[8]
	Acidic hydrolysis: 3% (v/v) HCl, 121°C autoclaving for 90 min Enzymatic: α-amylase (50 U/g food waste), pH 4.8, 30°C, 4 h	10 (% w/v)	<i>Kluyveromyces marxianus</i> and <i>Pichia kudriavzevii</i>	SHF: pH 4.8, 35°C, 5% inoculum, 48 h, 150 rpm	15.44–15.62 g L ⁻¹	[6]
	Acid: 1g HCl/100 g, at 121°C for 20 min; Enzymatic: α-amylase (50 U/g starch), pH 4.8, 50°C, 24 h	10–30 (% w/v)	<i>Kluyveromyces marxianus</i> , <i>Pichia kudriavzevii</i> , and <i>K. marxianus</i>	SHF: 30°C, 150 rpm, 72 h	42.19–83.30 g L ⁻¹	[1]
	Acid: 1.5% HCl (v/v), 90°C, 3h; Enzymatic: glucoamylase 85 U/mL, pH 5.0,, 60°C, 6h	1720 g wet weight/L	<i>S. cerevisiae</i>	SHF: 30°C, 120 rpm, pH 6.5-7.0, 10% (v/v) inoculum, 24 h	10.92 g L ⁻¹	[7]
	Enzymatic: crude fungal enzyme cocktail, 60°C, 500 rpm, pH 4.0-4.5, 24 h	127 g/L glucose	<i>S. cerevisiae</i>	SHF: 30°C, 100 rpm, 10% (v/v) inoculum, 32 h	58 g L ⁻¹	[38]
	Acidic hydrolysis: autoclave; citric acid (3% w/v), 130°C, 40 min	20 (% w/v)	<i>S. cerevisiae</i>	SSF: amylase (10 U/g waste), pH 5.5, 10% (v/v) of yeast suspension, 37°C, 150 rpm, 24 h.	54.04 g L ⁻¹	This study
Damaged maize grains	Gelatinization: autoclave at 15 psi for 1 h	14 (% w/v)	<i>S. cerevisiae</i>	SSF: pH 5.6, 31°C, 48 h; fungal amylase	42.4 g L ⁻¹	[9]
	Liquefaction: 3% (v/v) crude α-amylase (8.83 IU/mL), 65°C, pH 5.5, 2.5 h	12 (% w/v)	<i>S. cerevisiae</i>	SHF: 28°C, pH 5.5, 7.5% (v/v) inoculum, 24 h	2.9% (v/v)	[10]

Biomass	Pretreatment	Substrate loading	Microorganism	Fermentation conditions	Ethanol yield	Reference
	Saccharification: 1.5% (v/v) glucoamylase (27.71 IU/mL), 37°C, pH 5.5, 48 h					
	Liquefaction: 3% (v/v) crude α -amylase (8.83 IU/mL), 65°C, pH 5.5, 2.5	12 (% w/v)	<i>S. cerevisiae</i>	SSF: 1.5% (v/v) glucoamylase; 28°C, pH 5.5, 24 h	3.6% (v/v)	
	Acidic hydrolysis: autoclave; citric acid (3% w/v), 130°C, 40 min	20 (% w/v)	<i>S. cerevisiae</i>	SSF: amylase (10 U/g waste), pH 5.5, 10% (v/v) of yeast suspension, 37°C, 150 rpm, 24 h.	50.18 g L ⁻¹	This study
Damaged wheat grains		25 (% w/v)	<i>S. cerevisiae</i>	SSF: 6% inoculum; pH 5.8, 35°C, 200 rpm, crude bacterial amylase (10 mL, 44 U/mL), 0.3% peptone, 0.1% KH ₂ PO ₄ , 0.1% (NH ₄) ₂ SO ₄ , 80 h	4.4% v/v	[39]
	Thermal treatment (90°C, 30 min, water 1:5 w/v) + enzymatic liquefaction (α -amylase, 65°C, 90 min, pH 6–6.5) + saccharification (glucoamylase + xylanase/cellulase complex, 55–60°C, 120 min, pH 5–6)	1: 5 (w/v)	<i>S. cerevisiae</i>	SHF: 30–33°C; 72 h	69 g L ⁻¹	[40]
	Acidic hydrolysis: autoclave; citric acid (3% w/v), 130°C, 40 min	20 (% w/v)	<i>S. cerevisiae</i>	SSF: amylase (10 U/g waste), pH 5.5, 10% (v/v) of yeast suspension, 37°C, 150 rpm, 24 h.	52.18 g L ⁻¹	This study

Biomass	Pretreatment	Substrate loading	Microorganism	Fermentation conditions	Ethanol yield	Reference
Damaged sorghum grains		25 (% w/v)	<i>S. cerevisiae</i>	SSF: 6% inoculum; pH 5.8, 35°C, 200 rpm, crude bacterial amylase (10 mL, 44 U/mL), 0.3% peptone, 0.1% KH_2PO_4 , 0.1% $(\text{NH}_4)_2\text{SO}_4$, 80 h	3.5% v/v	[39]
	Gelatinization: autoclave 121°C, 30 min	2–12% starch	Co-culture: <i>Aspergillus niger</i> + <i>S. cerevisiae</i>	SSF; 30°C, pH 5, ~ 70 h, 150 rpm	4.2–22.2 g L^{-1}	[46]
	Acidic hydrolysis: autoclave; citric acid (3% w/v), 130°C, 40 min	20 (% w/v)		SSF: amylase (10 U/g waste), pH 5.5, 10% (v/v) of yeast suspension, 37°C, 150 rpm, 24 h.	64.39 g L^{-1}	This study
Waste potato biomass	Liquefaction: α -amylase (1 mL, 18.8 mg protein/mL) at 95°C, 3 h, pH 6.5 Saccharification: amyloglucosidase (0.8 mL, 300 U/mL) at 60°C, 72 h	16.16 (% w/v)	<i>S. cerevisiae</i>	SHF: 3% inoculum, 30°C, pH 5.5, 400 rpm, 48 h, with different nitrogen sources	30.8–35 g L^{-1}	[41]
	Enzymatic hydrolysis: α -amylase (590 U/g dry substrate) at 95°C, 3h; amyloglucosidase (25 U/g dry substrate) at 60°C, 72 h, 150 rpm	40.4 g/L	<i>S. cerevisiae</i>	SHF: 3% inoculum, 30°C, 150 rpm, 48 h, malt extract 50 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 4.84 g/L	24.6 g L^{-1}	[42]
	No pretreatment	92.37 g/L	co-culture <i>A. niger</i> + <i>S. cerevisiae</i>	SSF: 30°C, 150 rpm, 120 h, malt extract 59.42 g/L + $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.159 g/L	35.19 g L^{-1}	[11]

Biomass	Pretreatment	Substrate loading	Microorganism	Fermentation conditions	Ethanol yield	Reference
	Acidic hydrolysis: autoclave; citric acid (3% w/v), 130°C, 40 min	20 (% w/v)	<i>S. cerevisiae</i>	SSF: amylase (10 U/g waste), pH 5.5, 10% (v/v) of yeast suspension, 37°C, 150 rpm, 72 h.	52.38 g L ⁻¹	This study
Waste sweet potato	Acidic hydrolysis: autoclave (1.5% v/v HCl, 121°C, 15 min	15 (% w/v)	<i>S. cerevisiae</i>	SHF: 30°C, pH 5.0, 24 h	17.2–38.6 g L ⁻¹	[43]
	Acidic hydrolysis: autoclave; citric acid (3% w/v), 130°C, 40 min	20 (% w/v)	<i>S. cerevisiae</i>	SSF: amylase (10 U/g waste), pH 5.5, 10% (v/v) of yeast suspension, 37°C, 150 rpm, 72 h.	66.74 g L ⁻¹	This study

The ethanol conversion yields ranged from 122.54 to 333.69 g kg⁻¹ biomass, demonstrating substantial variability among the investigated substrates. The highest conversion efficiencies were generally associated with substrates possessing high starch availability and efficient sugar utilization during fermentation. These values are comparable to or higher than those reported for several agricultural and food-processing wastes used for bioethanol production, confirming the suitability of food waste streams as sustainable feedstocks within a circular bioeconomy framework [8, 44, 45]. In overall, these findings demonstrate that citric acid pretreatment combined with SSF provides an efficient and environmentally friendly strategy for converting diverse starchy food wastes into bioethanol, with substrate-specific fermentation kinetics influencing the optimal production time.

Conclusion

The present study demonstrated that mild citric acid pretreatment is an effective and sustainable strategy for enhancing bioethanol production from heterogeneous starchy food wastes. Among the investigated pretreatment variables, temperature exerted the greatest influence on fermentable sugar production, while its positive interaction with citric acid concentration significantly improved starch hydrolysis. The optimized conditions (3% citric acid, 130°C, and 40 min) enabled efficient sugar release and, when integrated with simultaneous saccharification and fermentation using *Saccharomyces cerevisiae*, produced 54.04 g L⁻¹ ethanol from kitchen food waste within only 24 h. Although sulfuric acid generated higher reducing sugar concentrations, citric acid pretreatment resulted in superior ethanol productivity because of lower inhibitor formation, demonstrating the importance of hydrolysate quality rather than sugar release alone. The optimized process was successfully applied to diverse starchy food wastes, yielding up to 66.74 g L⁻¹ ethanol and confirming its applicability to a wide range of waste substrates. Furthermore, the recovery of

approximately 92% of the applied citric acid substantially improves process sustainability and reduces chemical consumption, supporting the development of a near closed-loop pretreatment system. Overall, the integration of recyclable organic acid pretreatment with simultaneous saccharification and fermentation provides a practical, environmentally benign, and economically feasible platform for valorizing starchy food wastes into renewable bioethanol, contributing to waste management and the development of circular bioeconomy and sustainable biorefinery technologies.

Declarations

Conflicts of Interest

The authors declare no conflicts of interest.

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Author Contribution

M.H.: Conceptualization, Supervision, Funding acquisition, Project administration, Writing—original draft, Writing—review and editing; E.A.H.: Formal analysis, Writing—original draft, Writing—review and editing; N.A.N.: Conceptualization, Formal analysis, Writing—original draft, Writing—review and editing; M.G.: Conceptualization, Formal analysis, Writing—original draft, Writing—review and editing; A.K.: Methodology, Data curation, Formal analysis, Writing—original draft.

Data Availability Statement

The original contributions presented in this study are included in the article.

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Figures

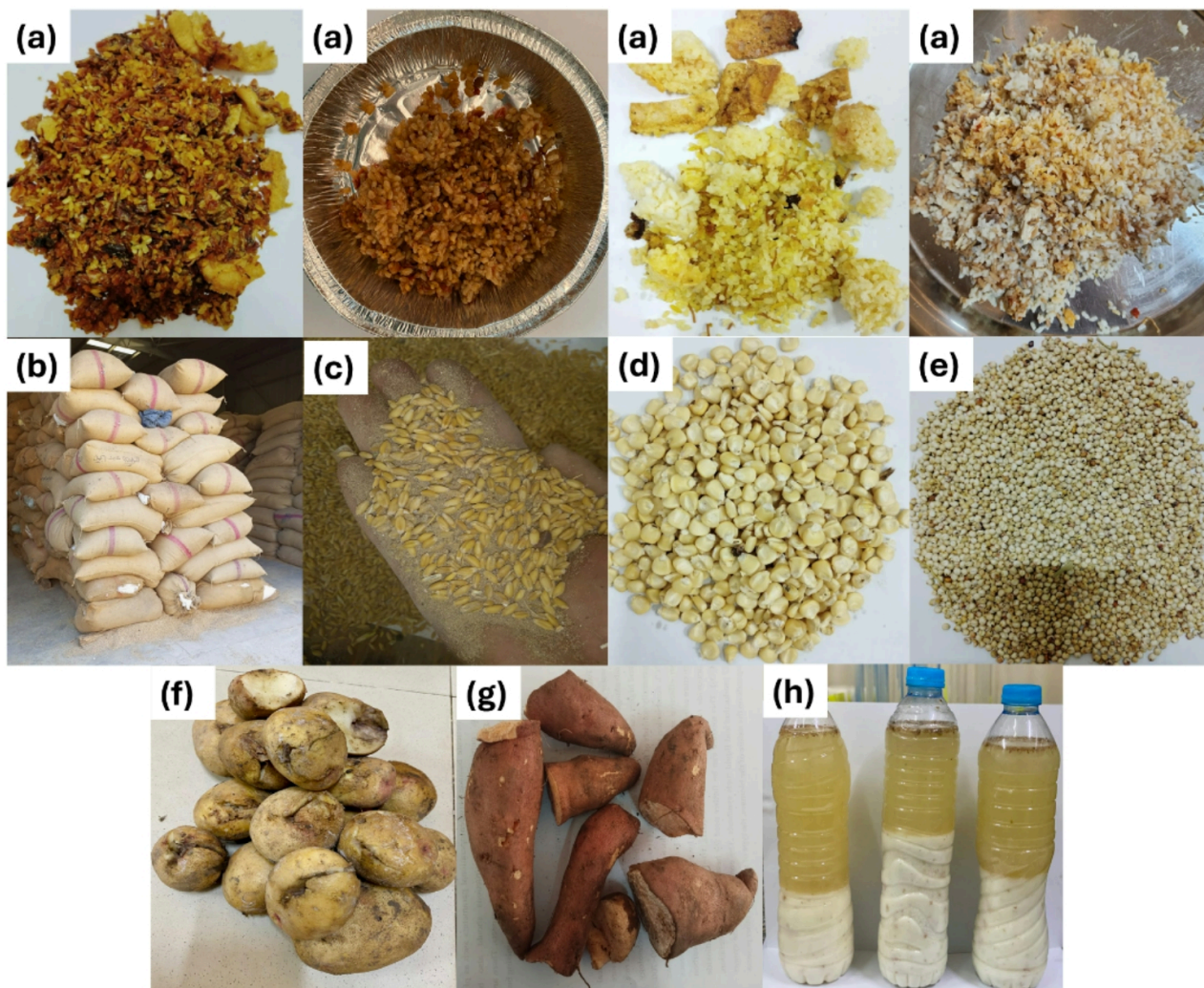


Figure 1

Photographs of collected starchy food waste. (a) kitchen leftover starchy waste, (b) rejected lots of grains, (c) damaged wheat grains, (d) damaged corn grains, (e) damaged sorghum grains, (f) infected potato tubers, (g) damaged sweet potato roots, (h) potato-processing wastewater stream generated during the washing stage at a potato chips factory.

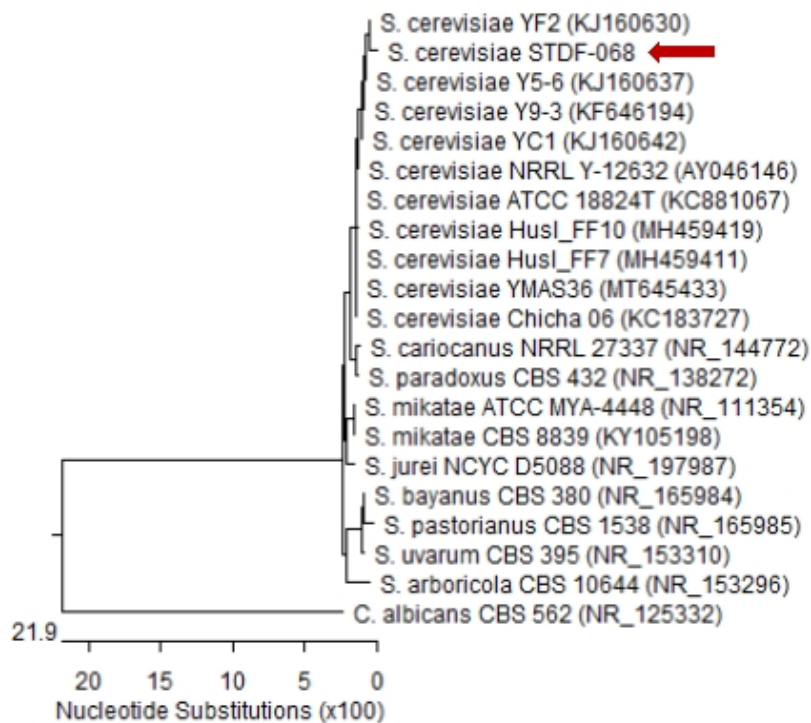


Figure 2

Phylogenetic tree based on ITS sequences of rDNA of *Saccharomyces cerevisiae* (isolate STDF-068, arrowed) aligned with closely related sequences accessed from the GenBank. This strain showed 99.28% - 100% identity and 88% - 97% coverage with several similar strains at NCBI including the type material *S. cerevisiae* strain ATCC 18824 (accession: AY046146). *Candida albicans* represents the outgroup strain. [C.= *Candida*, S.= *Saccharomyces*].

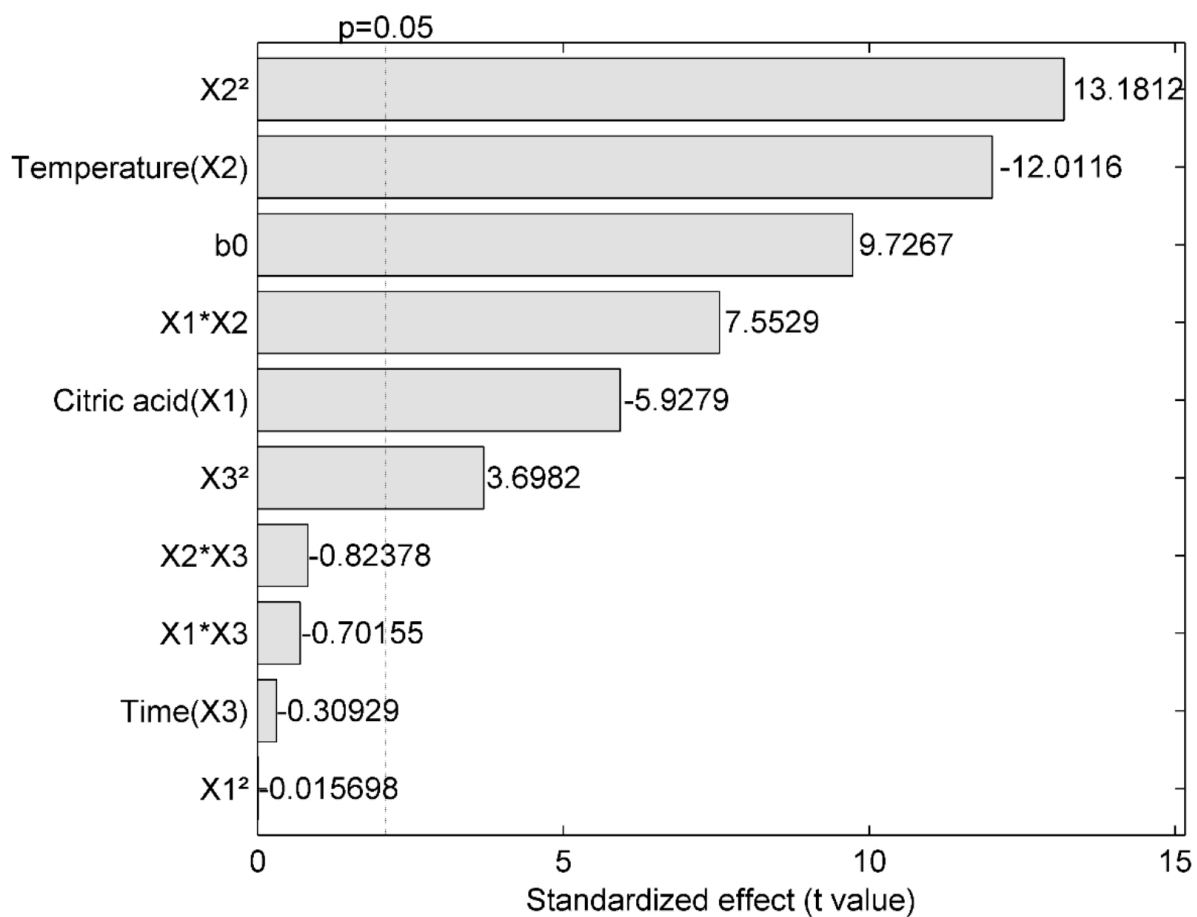


Figure 3

Pareto chart for the study of the effects of the variation in the parameters on the production of reducing sugars from starchy food waste.

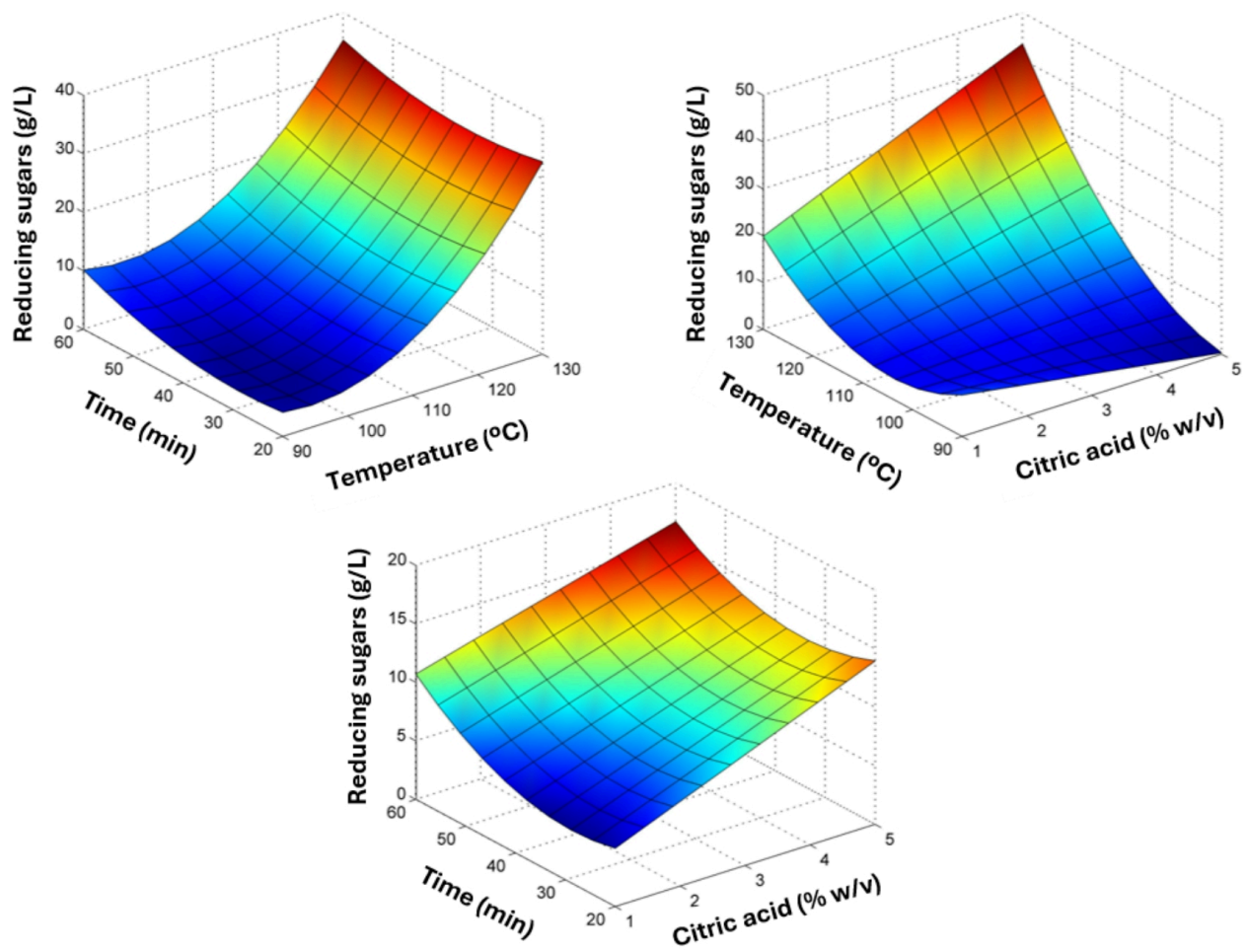


Figure 4

Response surface obtained by adjusting the data with the quadratic model and influence of the parameters in the production of reducing sugars from starchy food waste.

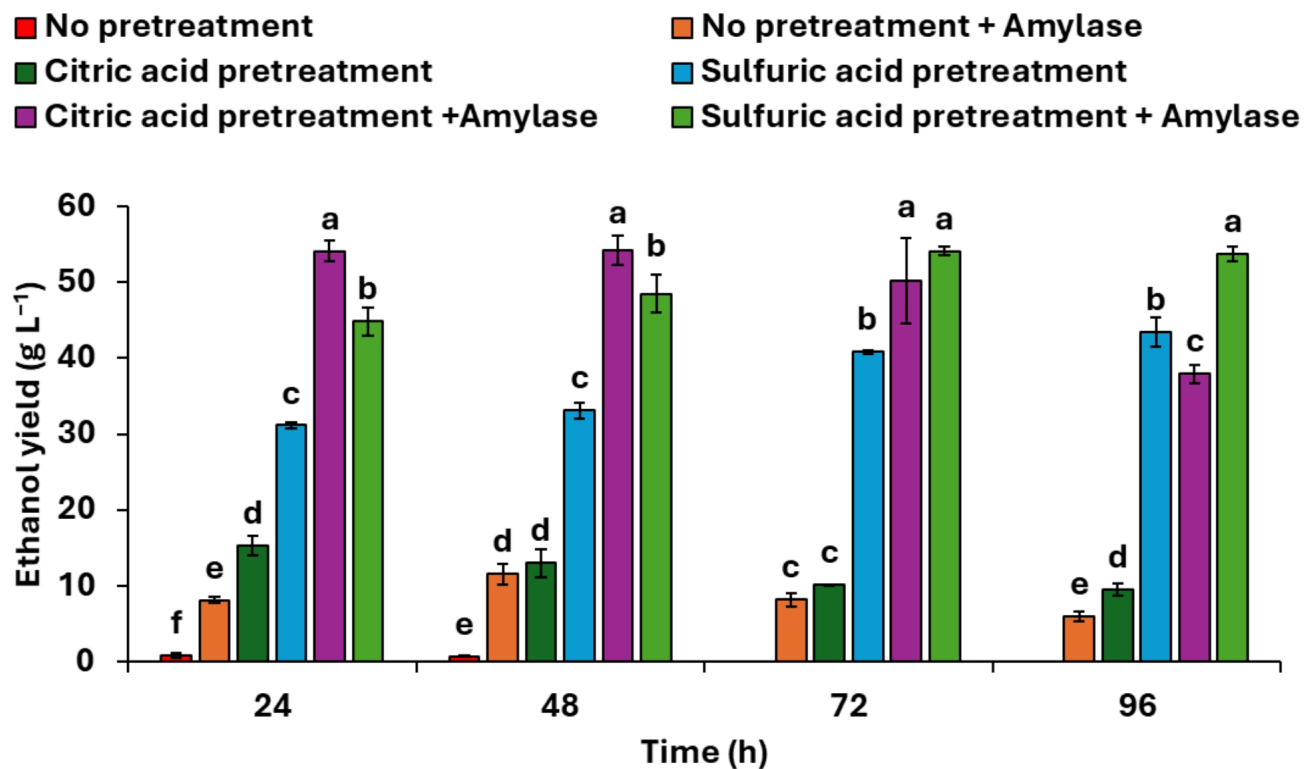


Figure 5

Variation of bioethanol yield from starchy food waste (kitchen leftover food waste) subjected to different pretreatment conditions with or without amylase supplementation in the fermentation medium at different culture periods. Values represent mean $\pm$ standard deviation of triplicate experiments. Different letters above columns indicate significant differences ($p < 0.05$) between different treatments at the same time

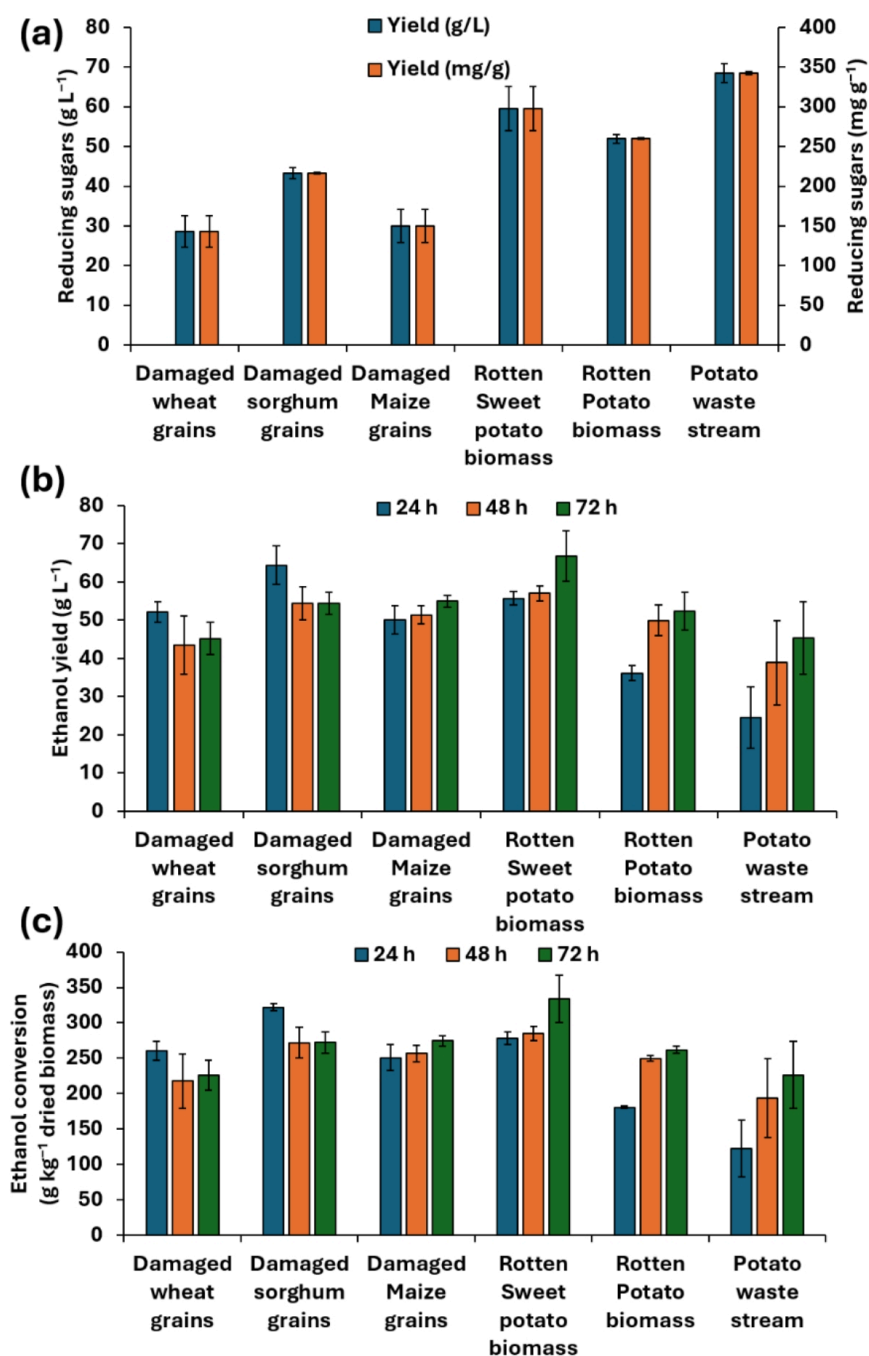


Figure 6

(a) Concentration of reducing sugars produced from 3% citric acid pretreatment of different wastes at 130 °C for 40 min. (b) Variation in bioethanol production from different starchy food wastes subjected to citric acid pretreatment followed by fermentation with amylase supplementation at different fermentation periods. (c) Ethanol conversion yields on a weight basis. Values represent mean $\pm$ standard deviation.