Egypt. J. Chem. **Vol. 67,** No. 10 pp. 211 - 222 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Biofuel production from pasta cooking wastes using Saccharomyces cerevisiae

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Abstract
Every kilogram of pasta produced throughout the production process produces roughly 23g of pasta waste (PW). Considering the manufacturing of pasta worldwide, over 376 billion tons of PW are produced each year. The process of producing bioethanol by employing the *Saccharomyces cerevisiae* strain to ferment carbohydrates recovered from cooked pasta leftovers that are not edible was investigated. The strains were cultured at pH 5.5–6.35 and 30°C with a sugar content of 100 g L-1. The obtained results demonstrated that the tested variety could produce ethanol from liquid kitchen pasta wastes (V/V) with concentrations ranging from 1.97 to 2.91 g/100 ml and efficiency ranging from 95.40% to 98.85%. Ethanol was created from solid kitchen pasta wastes at concentrations ranging from 2.26 to 2.45 g/100 ml and efficiency levels ranging from 92.96% to 94.72%. Additionally, the yeast strains were able to tolerate ethanol concentrations up to 18% at pH 3.8 and 37°C.

Keywords: Bioethanol, Saccharomyces cerevisiae, Pasta wastes, fermentation ;

1. Introduction

The fundamental component of economically viable, ecologically responsible, and sustainable electricity generation is renewable energy, or RE. According to an official report published by the International Energy Agency (IEA), the need for fossil fuels to create power has begun to decline since 2019 as renewable energy sources have become more prevalent in meeting the world's energy needs [1]. The technology that could be adopted for alternate energy sources will determine how these issues are resolved. Since at least the 1970s, when oil supplies were depleted and researchers started looking for other energy sources to replace petrol, small amounts of ethanol have been added to petrol. When it came to adding alcohol to petrol, ethanol was initially seen as the most appealing option. In contrast to petrol fuel, which is a nonrenewable energy source, ethanol may be produced from waste materials or natural goods. The fact that methanol and ethanol can be used without the engine's structure needing to be significantly altered is a noteworthy aspect [2]. Because it may be mixed with petrol and utilized as clean alcohol in engines with higher octane numbers and heat of vaporization, ethanol is a desirable alternative fuel. The production of bioethanol has surged quickly as a result of numerous nations' efforts to lower their reliance on foreign oil, support rural economies, and enhance air quality. Energy crops and lignocellulosic biomass are viable sources of ethanol for fuel [3]. The complexity of the manufacturing process is dependent on the kind of feedstock used. Thus, the design and execution technologies progress from a straightforward fermentation process for converting sugar to a multi-stage process for converting biomass to ethanol [4]. Several reviews, specifically on the manufacture of gasoline-ethanol from lignocellulosic biomass, have been published. Among the different substrates researched and tested, food waste is the most abundant, economical, and ubiquitously available substrate that can be utilized for bioethanol production. Annually around 1.3 billion tons of food is wasted globally, resulting in the wastage of land, water, energy, and input resources used for food production, leading to an economic loss of approximately 3.3 trillion USD [5]. As per the FAO's 2022 report, 3.1 billion people do not have access to a healthy diet, and the number of people affected by hunger increased from 150 million in 2019

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Receive Date: 21 December 2023, Revise Date: 25 February 2024, Accept Date: 04 April 2024
DOI: 10.21608/ejchem.2024.256272.9011

to 828 million in 2021, and yet such huge amounts of food are wasted [6]. Pasta is becoming a more and more popular cuisine around the globe, and several recipes have been created to enhance its nutritional value. Semolina, with its high gluten and protein content, is known to be the best raw ingredient for making traditional dry pasta. Optimizing the designs of the manufacturing process when using different raw materials requires a thorough understanding of the relationship between processing factors and pasta quality [7]. One of the most important basic foods, especially in developing nations, is pasta. To meet the need for food, the pasta business has continued to grow as a result of the exponential rise in the human population. The release of wastewater from the pasta industry into the surrounding water bodies and environment is a significant risk, necessitating the development of an environmentally friendly treatment strategy [10]. Pasta water waste, or the liquid that remains after cooking pasta, is an alternate starch substrate that can be utilized because starch is a common and easily accessible source of energy [9]. The starch concentration of pasta water waste—which is dependent on pasta quality—is the most significant criterion for the suitability of bioethanol production. Food wastes were fermented into ethanol using yeast after being subjected to a two-step enzymatic digestion procedure involving the use of alpha-amylase and gluco-amylase to break down starch [9]. Significant levels of carbohydrates (>50%) present in the residues of various agri-food wastes can be transformed into bioethanol [10]. Waste from cooked pasta can be used to produce bioethanol. A variety of biotechnological techniques were used to produce bioethanol. The three main processes are fermentation, distillation, and hydrolysis. The biomass's cellulose components are hydrolyzed to produce sugar, which is then fermented by microbes to produce alcohol [10]. Microorganisms meet their energy demand by converting carbon sources to by-products such as carbon dioxide, lactic acid, ethanol, etc. *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Kluyveromyces* spp., and *Schizo-saccharomyces pombe* are microorganisms able to convert sugars to ethanol. Yeasts are the most common microbial agents used for fermentation [11]. Finally, bioethanol is recovered from the extracts through distillation. The fermentation process converts glucose ($C_6H_{12}O_6$) or sugar into alcohol (CH_5OH) and carbon dioxide (CO_2) with the help of microorganisms such as yeast. The main objective of this study is the production of bioethanol from pasta-cooked wastes using the strain *Saccharomyces cerevisiae* yeast.

2. MATERIALS AND METHODS

Agro-industrial wastes:

Pasta kitchen wastes (PKW): Samples of pasta kitchen wastes (PKW)) were obtained whereas the samples of pasta were collected from the open market on 15th May, Helwan Governorate, Egypt. Pasta kitchen waste (PKW) was cooked and then dried at 55°C for 24 hours using a cross-flow drier. The dried pasta kitchen waste (PKW) was powdered using a hammer mill and passed through a 0.5mm sieve to obtain a fine powder. The samples were stored in the freezer until use. In the present work, the examined fresh Pasta kitchen wastes (PKWs) contained 85% water.

Waste pretreatments (Saccharification of raw materials)

Pasta kitchen wastes (PKW)samples (5 and 10g) were suspended in 100ml aliquots of either water or dilute H_2SO_4 and heat-treated at different temperatures for different periods (i.e.121°C for 10 minutes and 100°C for 30 minutes). Chemical treatment was applied using 1% H_2SO_4. Total sugars in the waste hydrolysates were measured using the phenol sulfuric acid method [12].

Isolation and Identification of Yeasts

The isolate of saccharomyces spp. was isolated using cells pre-culture medium (YP) composed of yeast extract 0.3%; beef extracts 0.5%; peptone 0.5%; glucose 1%; agar 2%; water 1000ml and pH5.5. The medium was sterilized at 121°C for 20min and was used in different batch fermentation experiments for ethanol production according to [13] from samples collected from source by active dry yeast from October City, Giza, Egypt. Isolate that showed high ethanol productivity were identified by studying specific morphological characteristics according to [14] and biochemical characteristics according to [15] and [16] at the Microbiology Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. The molecular tests were carried out at the Genetic Engineering and Agriculture Biotechnology Dept., Faculty of Agriculture, Ain Shams University, Qalyubia Governorate, Egypt. The strain was grown and maintained on YPDA medium.

a. Morphological characteristics

The methods used for the identification of the isolate were carried out according to the conventional ones based on morphological and physiological properties. The cultural characteristics including color, texture, appearance, elevation, and margin of colony were examined. Microscopical characteristics including cell shape, bud formation, presence of true mycelium, and fragmenting were also examined.[17]

b. Biochemical characteristics

Assimilation of various carbon compounds (D-glucose, D-galactose, D-xylose, L-arabinose, sucrose, maltose, cellobiose, lactose, raffinose, soluble starch, erythritol, mannitol, fermentation of glucose and cycloheximide 100ppm) were carried out according to

[18]. A separately sterilized solution of the tested nitrogen compounds (potassium nitrate, sodium nitrite, ethylamine-HCl, L-lysine-HCl, cadaverine-HCl, creatine, creatinine, D-glucosamine, imidazole, D-tryptophan and ammonium sulfate) were added aseptically to test tubes with 2.5ml sterile yeast carbon base and sterilized at 121°C for 20min [16]. Malt extract broth medium (5%) was inoculated with isolated strain and incubated at 25°C for 3 days. The cell was then microscopically examined for bud formation according to [18]. Ascospore formation was studied on McClary´s acetate agar medium (2.5% yeast extract, 1% dextrose, 10% potassium acetate, agar 3%, and pH 6.5) according to [19]. The isolated yeast strain was streaked on agar plates at 25, 28, 30, 35, 37, 40, 42, and 45°C for 48 hours to test the effect of temperature on the growth according to [18]. The ethanol tolerance of yeast strain was tested by inoculating a 5% broth culture of strain in an Erlenmeyer flask with YEPD broth containing 1% to 18% alcohol (v/v) in triplicates. After inoculation, the flask was incubated at 30°C for 48 hours. The sample was taken every 24 hrs. and optical density was recorded at 600nm using a spectrophotometer.

c. Molecular identification

1. Isolation of yeast genomic DNA

DNA isolation was performed by the modified method of (Hoffman and Winston, 1987).[20]

2. Molecular identification (PCR-FSP)

A preliminary grouping of isolates was performed based on fragment size polymorphism (FSP) of both the internal transcribed spacer (ITS1) and (ITS2) regions in rDNA using PCR [21]. The forward ITS1 primer (ITS1f) (5'-TCCGTAGGTGAACCTGCGG –3') and reverse ITS1 primer (ITS1r) (5'–GCTGCGTTCTTCATCGATG C –3') were used to amplify the ITS1 region. The forward ITS4 primer (ITS4f) (5'–GCATCGATGAAGAACGCAGC –3') and the reverse ITS4 primer (ITS4r) (5'–TCCTCCGCTTATTGATATGC –3') were used for amplification of ITS4 region, respectively. This primer was obtained from [22]. The amplicon of the region for individual yeast isolates was mixed and subjected to 1.5% agarose gel Electrophoresis, stained with ethidium bromide, and visualized under UV light. The molecular size of the DNA fragment was judged in comparison with the molecular standard 1KbDNAn ladder. The identification of yeast was based on the electrophoretic pattern for species.

3. PCR conditions

The conditions of PCR amplification were: 7min at 94°C, followed by 35 cycles to denaturing, 45sec. at 94°C, 1min at 56°C annealing, and 1min at 72°C with a final extension of about 7min at 72°C [22]. ITS4 region included (ITS4f and ITS4r) and the total region from ITS1f forward primer and ITS4r reverse primer was used, conditions of PCR amplification were 95°C for 120sec followed by 35cycles to denaturing at 95°C for 30sec., annealing, at 55°C, 30sec and 72°C for 60sec, with a final extension of about 10min at 72°C [23].

4. Purification of PCR products

Amplified products for S. cerevisiae isolate were purified using EZ-10 spin column PCR product purification. The PCR reaction mixture was transferred to a 1.5ml microfuge tube and three volumes of binding buffer 1 were added, then the mixture solution was transferred to the EZ-10 column and let stand at room temperature for 2 minutes. followed by centrifuging 750ul of wash solution was added to the column and centrifuge at 10.000rpm for 2min., repeated washing, at 10.000rpm was spine for an additional minute to remove any residual wash solution. The column was transferred into a clean 1.5ml microfuge tube and 50ul of elution buffer, incubated at room temperature for 2 minutes, and stored purified DNA at 20°C. [24]

5. ITS-sequencing and phylogenetic analysis

The representative isolate of yeast was selected for sequencing and phylogenetic analysis. A part of the rDNA region ITS1- 5.8S rDNA- ITS2 was amplified using the forward (ITS1) and reverse (ITS4) primer pairs. The sequencing of the product PCR was carried out using an automatic sequencer ABI PRISM 3730XL Analyzer using a big dye TM terminator cycle sequencing kite following the protocols supplied by the manufacturer. Single-pass sequencing was performed on each template using ITS1f- ITS4r primer. The fluorescent-labeled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems).[21], [22] and [24]

6. Computational analysis (BLASTn) and construction of a phylogenetic tree:

The Sequence was analyzed using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). [25] Sequences were aligned using Align Sequences Nucleotide BLAST; a neighbor-joining phylogenetic tree was constructed using *Meg Align* software.[26]

Ethanol production

For evaluating the ethanol production capabilities of the examined yeast strain, a set of fermentation batches was carried out using the fermentation medium (0.5% $(NH_4)_2SO_4$; 0.3% yeast extract; 0.5% KH_2PO_4; 0.1 $MgSO_4$; 0.01% $CaCl_2$) with pasta kitchen waste (PKW) hydrolysate (OH) (resulting from hydrolyses of 10g pasta kitchen waste (PKW) in 100ml 1.0N H_2SO_4 at 121°C for 15min). Another set of fermentation batches was carried out to study the effect of the incubation period on ethanol production from hydrolysate obtained from either liquid or solid pasta kitchen waste (PKW) using the effective yeast strain. All batches were run in 250ml Erlenmeyer flasks containing 100ml of the examined fermentation medium [13] and inoculated with 10% of the examined yeast seed culture. In all batches, pH was initially adjusted at a value from 5 -

5.5. Flasks were incubated at 30°C for 48 hours. Samples were periodically withdrawn at 24hrs–intervals to determine ethanol production kinetics. Ethanol yield was calculated according to the following equations:

Ethanol yield (%) = ethanol (g/100ml) / consumed sugar (g/100ml) × 100, (1a)

Efficiency from the theoretical yield (%) = [ethanol (g/100ml) / consumed sugar (g/100ml) × 0.511] × 100] (Sarabana,2006), (1b)

*Where sugar is interpreted as glucose in the fermentation work.

Biomass determination

Growth of the yeast strain (dry weight) was monitored according to the method described by [27].

Analytical Methods

Total sugars were determined using the phenol sulfuric acid method described by [12]. Ethanol was estimated according to Martin's method after being modified by [28], 1ml dichromate = 0.01g or 0.0126ml alcohol.

Statistical analysis

Statistical analysis was carried out using the *mSTAT* program. Variables having significant differences were compared using Duncan's multiple-range tests [29]. All experiments were replicated three times where data were presented as the means of three replicates.

Results and Discussion

1. **chemical composition of pasta kitchen wastes**

The percentage of the total protein, fat, total ash, crude fiber, starch, and total carbohydrates in dry pasta kitchen waste (PKW), were presefnted in (Table 1).

Table 1. Chemical composition of Pasta Kitchen waste (PKW)

Component	Content g/100g DW
Total carbohydrates	74.534
	74.638
	74.565
mean	**74.58**
Crud fibers	11.95
	12.01
	11.947
mean	**11.969**
Total Protein	12.65
	12.69
	12.7
mean	**12.68**
Lipids	0.274
	0.281
	0.285
mean	**0.28**
Total Ash	0.482
	0.476
	0.515
mean	**0.491**

* Values are the meaning of 3 replicates

2. Identification of yeast isolates

a. Morphological characteristics

The active isolate was sediment in broth culture, oval in cell shape, smooth in colony texture, whitish cream colony color, convex, no diffusion pigment, and mono-polar budding in asexual reproduction.

b. Biochemical and Physiological characteristics

Normally, the growth medium for S. cerevisiae is mainly carbon compounds that contain D-glucose, D-galactose, sucrose, maltose, raffinose, and soluble starch as carbon sources at 37°C. In the present study, to test the assimilation of different carbon sources, five of the twelve different sources of carbon were used instead of glucose (Table 2). Eleven different compounds were tested as a nitrogen source and only ammonium sulfate was used as a nitrogen source (Table 2). The obtained results confirmed that the isolate was S. cerevisiae. This finding agrees with that obtained by [30].

Table 2. Biochemical and physiological characteristics and assimilation of carbon and nitrogen compounds

Carbon compounds		Isolate reaction	Nitrogen compounds	Isolate reaction
D-lucose		+	Potassium nitrate	-
D-Galactose		+	Sodium nitrite	-
D-Xylose		-	Ethylamine-HCl	-
L-Arabinose		-	L-Lysine-HCl	-
Sucrose		+	Cadaverine-HCl	-
Maltose		+	Creatine	-
Cellobiose		-	Creatinine	-
Lactose		-	D-Glucosamine	-
Raffinose		+	Imidazole	-
Soluble starch		+	D-Tryptophan	-
Erythritol		-	Ammonium sulfate	+
Mannitol		-		
Nitrate		-		
Fermentation of glucose		+		
Max. growth T (^{o}C)		37		
Cycloheximide (100ppm)		-		

Heat tolerance of yeast strains

The strain has shown a similar growth pattern with good growth at 25°C to moderate growth at 30°C and 37°C, however, growth was inhibited at 40°C (Table 3). The same results were recorded by [31]. The inability of yeast isolates to grow at 42°C in the present study agrees

with the mesophilic characteristics of yeast reported in the literature [32].

Table 3. Effect of temperature on the growth of yeast

Isolates	Temperature (°C)						
	25	28	30	35	37	40	42
Yeast	++	++	++	++	++	±	-

* (++) Good growth; (+) Weak growth; (±) Feeble growth; (-) No growth

Ethanol tolerance

Generally, ethanol inhibits growth and is toxic to cells. As the concentration of ethanol increases in the medium, a reduction in growth is generally observed. In the present study, ethanol tolerance of a strain was recorded at a concentration of ethanol after which a sharp decrease in growth was observed. Variations in ethanol tolerance were observed among tested yeast strains (Table 4).

Table 4. Ethanol tolerance of yeast isolates

Ethanol Conc. (%)	1	2	3	4	5	6	7	**8**
Optical Density (OD)	1.91	1.87	1.83	1.78	1.73	1.68	1.64	**1.57**
Ethanol Conc. (%)	9	10	11	12	13	14	15	**16**
Optical Density (OD)	1.47	1.41	1.38	1.33	1.28	1.19	1.14	**1.09**

The strain showed the highest ethanol tolerance and was able to tolerate 18% ethanol concentration in the medium. At concentrations above 17%, a reduction in growth was observed with multiple drops of optical density values. It is worth mentioning that the reference strain (MTCC 170) was able to tolerate up to 12% of ethanol in the medium as at a concentration above 12%, a sharp decrease in growth was observed [12].

c. Molecular identification

Identification by sequencing analysis:

A neighbor-joining phylogenetic tree was constructed using *MegAlign* software [26] retrieved from the *Gene Bank* database and included in the analysis (Fig. 2).

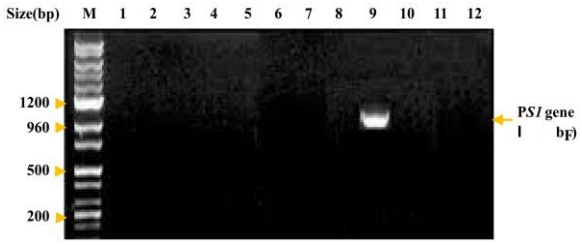
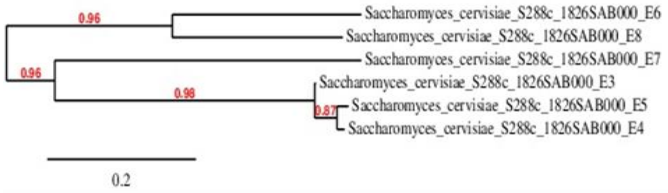
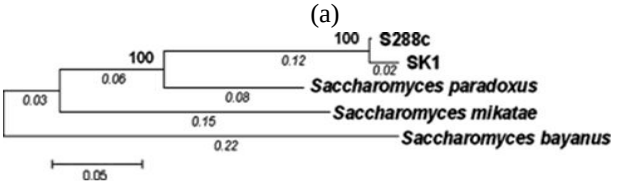


Fig.1. PCR for isolated yeast using primers ITS1 and ITS4. 1 Kb plus DNA Ladder (Invitrogen); isolated strain positive control (CK).



(a)



(b)

Fig.2. (a) The phylogenetic tree shows the relationship between S. sp. by forward primer. (b) Hierarchical clustering of S. cerevisiae S288c strain and four non-cerevisiae species. The analysis is based on sequence data from 77 different genes 460BP from each gene. A neighbor-joining tree was generated using the Kimura 2-parameter test as implemented in MEGA software version 2.1.

3. Production of ethanol by S. cerevisiae strain from different concentrations of glucose

Saccharomyces cerevisiae was tested for their ability to produce ethanol from 10% glucose (Table 5). This strain showed ethanol concentration (4.71g/100ml). However, as for ethanol production efficiency, the strain was (95.21%). The value of ethanol yield under present experimental conditions was 48.65%, which is close to the obtained yield recorded by [32] from 41.6 to 45.8% ethanol/g glucose.

Table 5. Production of ethanol by *S. cerevisiae* strain after 48 hours in batch fermentation containing different concentrations of glucose.

Conc. (%)	Yeast Dry Weight (g/10ml)	Consumed Sugar g/100ml	Ethanol kinetics production		
			g /100ml	Yield (%)	Efficiency (%)
1	0.238	0.94	0.42	44.68	87.44
2	0.317	1.81	0.83	45.86	89.74
3	0.391	2.37	1.09	45.99	90.00
4	0.417	3.17	1.47	46.37	90.75
5	0.485	4.14	1.94	46.86	91.70
6	0.542	5.2	2.44	46.92	91.83
7	0.601	6.04	2.84	47.02	92.02
8	0.649	7.83	3.69	47.13	92.22
9	0.743	8.39	4.05	48.27	94.47
10	0.836	9.66	4.71	48.65	95.21

*The dry weight of yeast biomass at the beginning of the fermentation was 0.250 g/100ml medium. * Values are the meaning of 3 replicates

3. Production of ethanol using active strain

Production of ethanol from different concentrations of Pasta Kitchen waste (PKW) (5% and 10%) by two different heat treatments using the yeast strain is shown in (Table 6). Pasta Kitchen waste (PKW)10% treated at 121°C for 10 minutes showed the highest ethanol concentration and efficiency (3.28g/100ml and 99.27%) followed by the concentration 5% treated at 121°C for 10 minutes (1.46g/100ml and 98.54%). Pasta Kitchen waste (PKW) 10% treated at 100°C for 30 minutes produced a low amount of ethanol (2.21gm/100ml with efficiency of 74.76%). Ethanol yield under present experimental conditions ranged from 38.20 to 50.73%, which was close to the theoretical yield (41.6 and 45.8% of ethanol/g glucose) recorded by [32] and 51.1g/g glucose recorded by [33].

Table 6. The effect of temperature treatment total soluble sugar (*TSS*) and hydrolysis of wastes

Treat-ment	Waste Conc. (%)	Yeast Dry Weight (g/100 m)	Consumed Sugar (g/100 ml)	Ethanol kinetics production		
				Production (g/100ml)	Yield (%)	Efficiency (%)
121°C + 10 minutes	5%	1.912	3.84	1.46^{f} ±0.095	50.35	98.54
	10 %	1.093	6.51	3.28^{a} ±0.036	50.73	99.27
100°C + 30 minutes	5%	1.911	3.47	1.73^{e} ±0.010	49.67	97.2
	10 %	1.064	5.93	2.25^{d} ±0.001	38.20	74.76

*Dry weight of yeast biomass at the beginning of the fermentation was 0.238 g /100 ml medium

* Values are the mean of 3 replicates

4. Ethanol production from different concentrations of pasta waste by yeast strain

Production of ethanol from different concentrations of pasta wastes was examined using the strain of yeast (*S. cerevisiae*).

4.1. Ethanol production from different concentrations of pasta liquid waste

(Table 7) show that, the highest ethanol production was recorded by control (pasta liquid) 0%) (3.717g/100ml) followed by pasta liquid (10%) (2.441g/100ml). As for ethanol production efficiency, the highest value was recorded by pasta liquid (10%) (98.199%) followed by pasta liquid (5%) (97.643.36%). The lowest ethanol production pasta liquid (5%) (1.245g/100ml) and efficiency were recorded by control (96.851%).

4.2. Ethanol production from different concentrations of pasta waste

(Table 8) show that, the highest ethanol production was recorded by control treatment (0 pasta waste) (3.510g/100ml) followed by 10% pasta waste (2.279g/100ml) and 7.5% pasta waste (1.697g/100ml). As for ethanol production efficiency, the highest value was recorded by 10% pasta waste (92.95%) followed by control (91.42%) and 7.5% pasta waste (90.563%). The lowest ethanol production and efficiency were recorded by 2.5% pasta waste (0.596g/100ml and 89.714%) and 5% pasta waste (1.149g /100ml and 89.031%), respectively.

5. Production of ethanol from pasta waste treated by different concentrations of H_2SO_4 using the yeast strain

Production of ethanol from 10% of pasta waste by different treatments (0 (control), 0.5, 1, 1.5, and 2% H_2SO_4) using yeast strain.

5.1. Production of ethanol from pasta liquid waste treated by different concentrations of H_2SO_4 using *S. cerevisiae.*

Production of ethanol from 10% of pasta liquid waste by different treatments (0 (control), 0.5, 1, 1.5, and 2% H_2SO_4) using yeast strain is shown in (Table 9). Pasta liquid waste treated (1.5% H_2SO_4) showed high ethanol concentration and efficiency (2.91g/100ml and (98.85%) followed by waste treated (1% H_2SO_4) (2.68/100ml and 98%) then followed by waste treated (0.5% H_2SO_4) (2.63g/100 ml and (97.12%). Pasta liquid waste treated (0% H_2SO_4) showed the lowest amount of ethanol 1.97g/100ml and an efficiency of 95.4%. Ethanol yield under the experimental conditions ranged from 48.75 to 50.51%, which was close to the theoretical yield of 41.6 and 45.8% of ethanol per 1g of glucose according to [34], of ethanol per1g of glucose according to [34], and by [33] (51.1g/g glucose).

Generally, pasta liquid wastes treated (0% H_2SO_4) had lower ethanol yields in comparison with the other concentrations used. It is also evident that treatments of H_2SO_4 (1.5%, 1%, and 0.5%) had higher ethanol yields in comparison with the other concentrations used, which obtained 2.91, 2.68, and 2.63g ethanol/100ml, respectively.

5.2. Production of ethanol from pasta waste is treated with different concentrations of H_2SO_4 using *S. cerevisiae*.

Production of ethanol from 10% of pasta waste by different treatments (0 (control), 0.5, 1, 1.5, and 2% H_2SO_4) using yeast strain is shown in (Table 10). Pasta waste treated (1% H_2SO_4) showed high ethanol concentration and efficiency (2.45g/100ml and 94.72%) followed by waste treated (1.5% H_2SO_4) (2.36/100ml and 94%) then followed by waste treated (0.5% H_2SO_4) (2.32g/100ml and 93.63%). Pasta waste treated (0% H_2SO_4) showed the lowest amount of ethanol

2.26g/100ml and an efficiency of 92.96%. Also, ethanol yield under the experimental conditions ranged from 47.50.75 to 48.40%, which was close to the theoretical yield of 41.6 and 45.8% of ethanol per 1g of glucose according to [34], and (Roehr, 2001) (51.1g/g glucose). Generally, pasta waste control (0% H_2SO_4) had lower ethanol yields in comparison with the other concentrations used. It is also evident that concentrations of H_2SO_4 (1, 1.5, and 0.5%) had higher ethanol yields in comparison with the other concentrations used, which obtained 2.45, 2.36, and 2.32g ethanol/100ml, respectively. The durum wheat pasta is a staple food, comprising 74–77% carbohydrates and 11–15% protein, but lacks minerals, vitamins, essential amino acids and phenolic compounds [35]. Chemical and biological processes individually or in combination have been used widely and have been the method of choice for obtaining fermentable sugars. The dilute acid treatment followed by enzymatic treatment is the most sought and successful pretreatment method with comparatively less inhibitor formation than in concentrate acid pretreatment [36]. The dilute acid treatment changes the structural conformation, depending on the parameters (temperature, time, type of acid, and concentration), and it also increases the surface area accessibility of the substrate to aid better enzymatic hydrolysis and saccharification [37]. [38] optimized and scaled up the dilute acid fractionation of liquid and solid portions of the dried food waste, using sulfuric acid 0%, 0.4, and 0.8% (v/v) at a temperature of 130, 160, and 190°C for 1, 64.5 and 128min. The maximum glucose concentration (26.4g/L) was obtained from food waste treated with 0.37% (v/v) H_2SO_4 at 149.8°C for 123.6 minutes. [39], used dilute H_2SO_4 pretreatment followed by treatment with Cellic Ctec 2 to pretreat the food waste from MSW to produce hydrolysate with a sugar concentration of 25g/L [40]. The further fermentation of the hydrolysate with Mucor indicus gave an ethanol titer of 20g/L. Similarly, a higher sugar content was obtained after dilute acid treatment (HCL-33.7g/L, and H_2SO_4-40.5g/L) than with the hydrothermal treatment (27.6g/L) carried at T = 90°C before proceeding with the enzymatic hydrolysis [41]. Cooked pasta, a food reported to have a low glycemic index is generally described as a compact matrix with starch granules entrapped in a protein network [42] and [43]. Its structure, as well as its composition, are responsible for the specific nutritional properties of pasta among cereal products [44]. Indeed, the unique feature of pasta is that it contains slowly digestible starch [45] and [46]. Modifying either the manufacturing or cooking process parameters could affect the pasta structure and therefore potentially alter the digestibility of the starch and protein fractions [47].

In this study the yeast strain was isolated from an active dry yeast, and it was then identified morphologically and genetically. The best sugar concentration at which the yeast could efficiently produce the most ethanol was then determined by testing its ability to produce ethanol from glucose. The process of producing ethanol from food waste (pasta) was then evaluated, and steam pressure treatment was used to determine the waste's ideal concentration. To maximize the amount of simple sugars required for the yeast to carry out the fermentation process and raise the productivity of ethanol, various concentrations of concentrated sulfuric acid were added at the end to improve the efficiency of the digesting process.

Table 7. Ethanol production from different concentrations of pasta liquid waste

Treatments	pH		Dry Waste		Sugar (gm/100ml)		Ethanol		
	Zero	48hrs	zero	48hrs	Initial	Consumed	ml/100ml	Yield %	Efficiency %
Control	5.560	3.790	0.020	0.212	9.570	7.630	3.758	49.257	96.392
	5.560	3.790	0.020	0.213	9.720	7.320	3.715	50.749	99.313
	5.560	3.770	0.020	0.213	9.710	7.590	3.679	48.467	94.847
Mean	**5.560**	**3.783**	**0.020**	**0.213**	**9.667**	**7.513**	**3.717[a]±0.8313**	**49.491**	**96.851**
Pasta liquid 5%	5.700	3.400	0.030	0.110	3.590	2.530	1.274	50.375	98.581
	5.730	3.410	0.037	0.135	3.580	2.440	1.238	50.749	99.313
	5.640	3.520	0.036	0.134	3.750	2.520	1.224	48.563	95.036
Mean	**5.690**	**3.443**	**0.034**	**0.126**	**3.640**	**2.497**	**1.245[c]±0.2117**	**49.896**	**97.643**
Pasta liquid 10%	5.520	3.800	0.050	0.170	5.820	4.770	2.397	50.249	98.335
	5.530	3.670	0.049	0.172	6.310	4.920	2.448	49.748	97.354
	5.570	3.620	0.053	0.166	6.350	4.900	2.477	50.542	98.908
Mean	**5.540**	**3.697**	**0.051**	**0.169**	**6.160**	**4.863**	**2.441[b]±0.4497**	**50.180**	**98.199**

*Values are mean of 3 replicates and means showing the same letters are not significantly different (p≤0.05). S.E.±0.079.
Working volume = 100ml.

Table 8. Production of ethanol by S. cerevisiae in batch fermentation containing different concentrations of pasta waste.

Treatments	pH		Dry Waste		after 48hrs		Ethanol		
	zero	48hrs	zero	48hrs	Initial sugar (gm/100ml)	Consumed sugar (gm/100ml)	ml/100ml	Yield (%)	Efficiency (%)
Control	5.500	3.620	0.020	0.100	9.570	7.630	3.570	46.789	91.564
	5.530	3.600	0.020	0.110	9.720	7.320	3.440	46.990	91.957
	5.650	3.530	0.020	0.107	9.710	7.590	3.519	46.368	90.739
Mean	**5.560**	**3.583**	**0.020**	**0.106**	**9.667**	**7.513**	**3.51[a]±0.7850**	**46.716**	**91.420**
Pasta 2.5%	5.640	3.710	0.030	0.110	1.720	1.350	0.652	48.276	94.473
	5.640	3.690	0.037	0.115	1.740	1.340	0.630	47.015	92.006
	5.620	3.760	0.036	0.114	1.650	1.200	0.507	42.241	82.664
Mean	**5.633**	**3.720**	**0.034**	**0.113**	**1.703**	**1.297**	**0.596[e]±0.1349**	**45.844**	**89.714**
Pasta 5%	5.660	3.740	0.050	0.137	3.450	2.510	1.180	47.026	92.027
	5.660	3.790	0.049	0.132	3.420	2.480	1.137	45.843	89.712
	5.700	3.720	0.053	0.148	3.450	2.590	1.130	43.616	85.354
Mean	**5.673**	**3.750**	**0.051**	**0.139**	**3.440**	**2.527**	**1.149[d]±0.2570**	**45.495**	**89.031**
Pasta 7.5%	5.630	3.840	0.044	0.160	5.310	3.610	1.738	48.142	94.212
	5.720	3.840	0.045	0.163	5.230	3.520	1.579	44.847	87.764
	5.680	3.780	0.051	0.171	5.180	3.870	1.774	45.843	89.713
Mean	**5.677**	**3.820**	**0.047**	**0.165**	**5.240**	**3.667**	**1.697[c]±0.3804**	**46.277**	**90.563**
Pasta 10%	5.630	3.670	0.071	0.183	7.060	4.740	2.216	46.748	91.484
	5.690	3.620	0.074	0.192	6.940	4.790	2.267	47.318	92.600
	5.630	3.670	0.071	0.187	6.970	4.860	2.353	48.425	94.765
Mean	**5.650**	**3.653**	**0.072**	**0.187**	**6.990**	**4.797**	**2.279[b]±0.5098**	**47.497**	**92.950**

*Values are mean of 3 replicates and means showing the same letters are not significantly different (p≤0.05). S.E.±0.014.
Working volume = 100ml.

Table 9. Production of ethanol from pasta liquid waste by *S. cerevisiae* in batch fermentation using different concentrations of H_2SO_4.

Treatments	pH		Dry Waste		Sugar (gm/100ml)		Ethanol		
	zero	48hrs	zero	48hrs	Initial sugar	Consumed sugar	ml/100ml	Yield %	Efficiency %
Control	5.52	3.8	0.0509	0.27	5.94	4.1	2.03	49.45	96.78
	5.53	3.78	0.0492	0.26	6.42	3.99	1.96	49.00	95.89
	5.57	3.72	0.0516	0.26	6.21	4.06	1.94	47.80	93.54
Mean	**5.540**	**3.767**	**0.051**	**0.263**	**6.190**	**4.050**	**1.97^{d}±0.4417**	**48.75**	**95.40**
Pasta L. (0.5%H_2SO_4)	5.52	3.7	0.0531	0.24	6.13	5.16	2.56	49.68	97.22
	5.53	3.7	0.0487	0.22	6.41	5.37	2.66	49.49	96.85
	5.57	3.68	0.0522	0.21	6.52	5.39	2.68	49.71	97.28
Mean	**5.540**	**3.693**	**0.051**	**0.223**	**6.353**	**5.307**	**2.63^{b}±0.5891**	**49.63**	**97.12**
Pasta L. (1%H_2SO_4)	5.52	3.71	0.0541	0.17	6.17	5.32	2.65	49.82	97.49
	5.53	3.67	0.0492	0.19	6.48	5.39	2.71	50.25	98.33
	5.57	3.62	0.0489	0.196	6.55	5.34	2.68	50.17	98.19
Mean	**5.540**	**3.667**	**0.051**	**0.185**	**6.400**	**5.350**	**2.68^{b}±0.5992**	**50.08**	**98.00**
Pasta L. (1.5%H_2SO_4)	5.52	3.64	0.0519	0.192	6.37	5.58	2.82	50.61	99.04
	5.53	3.67	0.0512	0.182	6.61	5.77	2.91	50.45	98.73
	5.57	3.62	0.0523	0.186	6.75	5.91	2.98	50.48	98.79
Mean	**5.540**	**3.643**	**0.052**	**0.187**	**6.577**	**5.753**	**2.91^{a}±0.602**	**50.51**	**98.85**
Pasta L. (2%H_2SO_4)	5.52	3.69	0.0515	0.191	6.12	4.44	2.17	48.93	95.75
	5.53	3.67	0.0497	0.194	6.47	4.55	2.24	49.34	96.55
	5.57	3.72	0.0528	0.193	6.53	4.52	2.20	48.70	95.31
Mean	**5.540**	**3.693**	**0.051**	**0.193**	**6.373**	**4.503**	**2.21^{c}±0.4934**	**48.99**	**95.87**

*Values are mean of 3 replicates and means showing the same letters are not significantly different ($p \leq 0.05$). S.E.±0.006. Working volume = 100 ml.

Table 10. Production of ethanol from pasta waste by *S. cerevisiae* in batch fermentation using different concentrations of H_2SO_4

Treatments	pH		Dry W		T.S.S. (gm/100ml)		Ethanol		
	zero	48 hrs.	zero	48 hrs.	Initial sugar	Consumed sugar	ml/100ml	Yield %	Efficiency %
Control	5.52	3.843	0.0525	0.161	7.06	4.92	2.32	47.10	92.17
	5.53	3.846	0.0527	0.164	6.94	4.7	2.24	47.76	93.47
	5.65	3.781	0.0527	0.17	6.97	4.65	2.22	47.65	93.25
Mean	**5.567**	**3.823**	**0.053**	**0.165**	**6.990**	**4.757**	**2.26^{d}±0.5054**	**47.50**	**92.96**
Pasta (0.5%H_2SO_4)	5.64	3.743	0.0533	0.163	7.06	4.89	2.35	47.98	93.89
	5.64	3.794	0.0537	0.165	6.94	4.8	2.29	47.67	93.29
	5.62	3.727	0.0536	0.164	6.97	4.84	2.32	47.88	93.69
Mean	**5.633**	**3.755**	**0.054**	**0.164**	**6.990**	**4.843**	**2.32^{c}±0.5182**	**47.84**	**93.63**
Pasta (1% H_2SO_4)	5.66	3.719	0.0551	0.157	7.06	5.12	2.46	48.09	94.10
	5.66	3.699	0.0498	0.162	6.94	4.97	2.40	48.37	94.66
	5.7	3.762	0.0513	0.158	6.97	5.11	2.49	48.75	95.40
Mean	**5.673**	**3.727**	**0.052**	**0.159**	**6.990**	**5.067**	**2.45^{a}±0.5485**	**48.40**	**94.72**
Pasta (1.5% H_2SO_4)	5.63	3.675	0.0604	0.166	7.06	4.99	2.39	47.89	93.72
	5.72	3.626	0.0575	0.163	6.94	4.88	2.33	47.78	93.51
	5.68	3.687	0.0561	0.171	6.97	4.86	2.35	48.42	94.76
Mean	**5.677**	**3.663**	**0.058**	**0.167**	**6.990**	**4.910**	**2.36^{b}±0.5274**	**48.03**	**94.00**
Pasta (2% H_2SO_4)	5.63	3.532	0.0571	0.159	7.06	4.94	2.36	47.79	93.52
	5.69	3.541	0.0574	0.161	6.94	4.81	2.30	47.87	93.69
	5.63	3.539	0.0571	0.167	6.97	4.8	2.27	47.22	92.41
Mean	**5.650**	**3.537**	**0.057**	**0.162**	**6.990**	**4.850**	**2.31^{c}±0.5167**	**47.63**	**93.20**

*Values are mean of 3 replicates and means showing the same letters are not significantly different ($p \leq 0.05$). S.E.±0.0056. Working volume = 100ml.

5. Conclusions

Based on the above results and discussion it may conclude that strains of saccharomyces cerevisiae have the ability to produce ethanol from various carbon sources. A waste concentration of 10% was the most suitable condition for the work of yeasts. The best thermal treatment of pasta liquid wastes was at 121°C for 15 minutes and acid treatment by 1% H_2SO_4. The best thermal treatment of pasta wastes was at a temperature of 121°C for 15 minutes and acid treatment by 1.5% H_2SO_4. The best thermal treatment of wastes was at a temperature of 121°C for 15 minutes and acid treatment by 1.5% followed by 1% of H_2SO_4.

This shows that Pasta wastes can be a good carbon source for yeast during alcohol fermentation due to their contents of carbohydrates. This study needs more studies and future research to increase the utilization of organic waste, especially kitchen waste rich in carbohydrates.

6. Conflicts of interest

The authors confirm that there are no known *conflicts of interest* associated with this publication and there has been no significant *financial support* for this work that could have influenced its outcome.

The authors confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, concerning intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

The authors understand that the corresponding author is the contact for the editorial process and is responsible for communicating with the other authors about progress, submissions of revisions, and final approval of proofs.

7. Formatting of funding sources

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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