Article

Not peer-reviewed version

Alcoholic Fermentation of Tomato Canning Waste (TCW) from the Adrar Region in the Southwest of Algeria

Kamel Kaidi , Abdelaziz Arroussi , Mohammed Djaafri , Mostefa Khelafi , Ahmed Tahri , Fethya Salem , Slimane Kalloum , Abdulaziz E. Atabani , Arianna Baldinelli *

Posted Date: 16 December 2024

doi: 10.20944/preprints202412.1268.v1

Keywords: tomato cannery waste; fermentation; bioethanol; pretreatment; biowaste; bioenergy; biofuels; steam explosion; circular-economy; sustainable fuels

 doi:10.20944/preprints202412.1268.v1

Article

Alcoholic Fermentation of Tomato Canning Waste (TCW) from the Adrar Region in the Southwest of Algeria

Kamel Kaidi [1], Abdelaziz Arroussi [2], Mohammed Djaafri [3], Mostefa Khelafi [3], Ahmed Tahri [3], Fethya Salem [3], Slimane Kalloum [4], Abdulaziz E. Atabani [5] and Arianna Baldinelli [6,*]

[1] Laboratoire de Matériaux Catalytiques et Procédés Industriels (LMCPI), Département des hydrocarbures et énergies renouvelables, Faculté des Sciences et de la Technologie, University of Adrar, Adrar, Algeria
[2] Laboratory of Saharan Natural Resources, Faculty of Natural and Life Sciences, University of Adrar, Algeria
[3] Unité de Recherche en Energie Renouvelables en milieu saharien, URERMS, Centre de Développement des Energies Renouvelables, CDER, Adrar, Algeria
[4] Laboratory of Energy, Environment and Information System, Faculty of Material Sciences, Mathematics and Computer Science, University of Adrar, Algeria
[5] Green Technology Research Center, Yuan Ze University, Jhongli City, Taoyuan County 320, Taiwan
[6] Dipartimento di Ingegneria dell'Energia, dei Sistemi, del Territorio e delle Costruzioni (DESTEC), University of Pisa, Pisa, Italy
* Correspondence: arianna.baldinelli@unipi.it

Abstract: This research aims to promote bioethanol production for the first time from tomato waste (skins, pips and peels) generated by the "Fouggara" tomato canning factory in Réggane/Adrar, in South-West Algeria. The waste is thermally pre-treated, followed by chemical pre-treatment with sulphuric acid. This process is characterized by a drop in density from 1.14 to 1.03 kg/l accompanied by a significant release of carbon dioxide (CO_2) during alcoholic fermentation. The produced CO_2 mainly resulted by the glucose converting into ethanol followed by weight loss in the form of CO_2. A decrease in reducing sugars from 5.24 to 2.02 mg/L was recorded at the end of fermentation. Bioethanol production reaches an alcohol content of 12° in 72 hours, with a density of 0.886 kg/L, close to that of commercial ethanol, which is 0.789 kg/L.

Keywords: tomato cannery waste; fermentation; bioethanol; pretreatment; biowaste; bioenergy; biofuels; steam explosion; circular-economy; sustainable fuels

1. Introduction

Organic waste recovery as a viable sustainability practice for waste management and renewable energy production. Organic waste, comprising food scraps, yard waste, and agricultural byproducts, holds potential as a resource for energy generation and valuable product creation through methods such as composting, anaerobic digestion (AD), and pyrolysis. The recovery of organic waste is sustainable due to its ability to reduce greenhouse gas emissions (GHGs), particularly methane (CH_4) from landfill decomposition, and to serve as a renewable energy source that diminishes dependence on fossil fuels. Additionally, organic waste recovery promotes resource recovery within a circular economy by converting waste into compost and biofertilizers, enhancing soil health and reducing chemical fertilizer usage. It also reduces the burden on landfills, mitigating associated pollution and conserving valuable space. Economic advantages, including new revenue streams and job creation, further support its implementation [1]. In particular, organic agricultural waste presents a significant environmental challenge in the agricultural sector, highlighting the importance of valorizing this waste biomass for bioenergy production [2].

1.1. Tomato Waste

In 2021, the global tomato production reached 189 million metric tons exhibiting a growing trend (+4% compared to 2017), cultivated on a total area of 4.84 million hectares dedicated to this crop [3]. In the Adrar region (Southwest of Algeria), the cultivation of tomatoes (*Solanum lycopersicum* L.) has

 doi:10.20944/preprints202412.1268.v1

been introduced since 1970. Its production has experienced significant growth due to the expansion of cultivated areas, which increased from 50 hectares in 1970 to 1140 hectares in 2019 [4]. The composition of the tomato is influenced by the climate (humid or dry), temperature, soil type, agricultural practices, and the timing of planting and harvesting. Tomatoes in the Adrar province are of an early nature: they are planted outdoors in late August without the need for a greenhouse, and they are harvested in December. According to statistics from the Agricultural Services Directorate of Adrar for the year 2019, c.a. 693 tons of tomatoes produced in the Adrar region were destined for processing [5]. According to Shrestha et al., [6], for every kilogram of tomatoes processed, 40 g of by-products are generated, with 20 g being waste and 20 g being tomato pomace. Furthermore, del Valle et al., [7] reported that up to 4% of the total weight of processed tomatoes could be rejected after the canning process. The solid residue that remains after processing tomatoes to create tomato juice, paste, sauce, puree, or ketchup is commonly known as tomato canning waste (TCW) [8]. The dry pomace contains 44% seeds and 56% pulp and skin, while the wet pomace has 33% seeds, 27% skin, and 40% pulp [9]. TCW's chemical components differ according to the sample that is being examined. However, it frequently falls within these ranges: 12–23% protein, 5–20% fat, 4–6% ash, 27–32% cellulose, 5–18% hemicellulose, 11–26% simple sugars, 7.6% pectin, and 31% lignin [10–12]. Additionally, tomato waste contains some mineral salts, including smaller concentrations of iron, manganese, and copper (15–30 mg/kg) with high levels of calcium, potassium (about 7–11 g/kg), magnesium, sodium, and phosphorus (around 2-3 g/kg) [13].Tomato waste has previously been exploited as animal feed [14]. However, a research carried out by Heuzé et al. [15] in 2015 has demonstrated that the use of organic tomato waste as livestock feed is not beneficial due to its limited nutritional value. Locally, despite the large tomato production, around 90% of TCW in the Adrar region is thrown away untreated, with harmful consequences for the environment: infiltration of liquids into the soil, risk of groundwater pollution, foul odors and GHGs. In practice, there are a number of ways to treat organic waste, including AD, alcoholic fermentation (AF), composting, and incineration [16,17]. For the TCW, classic incineration is seen as an unprofitable and polluting method. Utilizing these waste materials as compost for fertilization is also considered unprofitable because TCW is characterized as acidic, which may require additional pre-treatments and post-treatments (such as grinding, mixing with other substances, sorting, etc.) [18,19].

A number of researches are currently focusing on the energy recovery from agricultural and agro-industrial wastes to produce biofuels [20,21]. AF to produce bioethanol is widely employed and can serve as a treatment solution for this particular agro-industrial waste, TCW. This process is utilized in the agri-food industry for fermented beverages such as wine, beer, and so on. It is also used in industrial ethanol production, particularly as fuel or a basic raw material in the chemical industry [22].

1.2. State of the Art Alcoholic Fermentation

Recent studies on AF of tomato waste focuses on improving fermentation conditions to increase ethanol production while efficiently using tomato by-products. Recent research has proven a variety of procedures and settings that enhance ethanol generation from tomato pulp and other waste sources. According to studies, the best fermenting conditions for tomato pulp include a sugar content of 230 g/L and an inoculum of 5 g/L, which yields roughly 101.5 g/L of ethanol with 99.5% efficiency [23,24]. Another study found that the fermentation process had sluggish kinetics, with maximal substrate consumption rates of 4.545 g/(L·h) and ethanol productivity of 1.39 g/(L·h). Further research has shown that even tomato waste could be converted to bioethanol, with maximum ethanol concentrations of 0.17% under the right conditions [25].

The AF of tomato waste is affected by many critical parameters, including inoculum, temperature, initial sugar concentration and pH levels. These factors significantly impact final product quality, including overall alcohol content, flavor profile and other sensorial characteristics. The ideal ratio of inoculum for fermentation is generally around 5% w/w [26]. Increasing inoculum concentrations may promote faster fermentation, but not necessarily improving final product quality [27]. For fermentation temperatures, the best results are achieved between 22 °C and 34.5 °C, since specific studies indicate that 31°C is an effective temperature for maximizing viable cell numbers [28]. As far as sugar is a key factor in alcohol yield, higher concentrations can increase ethanol production. Research confirms that sugar levels between 8.8% and 14.4% provide an optimum alcohol content of

 doi:10.20944/preprints202412.1268.v1

around 11%. In fact, tomato waste juice must be kept at a pH of around 3.7 to optimize fermentation conditions. However, optimizing these factors can improve bioalcohol quality from tomato waste, but variations in feedstock quality, such as tomato ripeness, can also lead to significant differences in the final product, suggesting that not all factors can be controlled or predicted [29].

1.3. Alcoholic Fermentation Principles

In this metabolism, organic molecules play the role of the final electron acceptor. Nicotinamide adenine dinucleotide, or NAD+, is utilized initially by yeast as an intermediate electron acceptor before being reduced to NADH (reduced nicotinamide adenine dinucleotide). The final reduction of acetaldehyde to CO_2 and ethanol contributes to the preservation of the redox equilibrium. The NADH generated during glycolysis process, the principal mechanism for metabolising sugars to pyruvate, plays a key role in the energy balance of this conversion, as described by Equation (1):

$$C_6H_{12}O_6 + 2\ Pi + 2\ ADP \rightarrow 2\ C_2H_6O + 2\ CO_2 + 2\ ATP \quad (1)$$

The nature of the acid is of great importance, as each yeast species requires an optimal pH for its growth. The acidic pH of TCW is detrimental to bacterial development but favorable for yeast and mold proliferation [30,31]. The optimal pH for yeast growth is typically between 4.3 and 4.7 [32], with *Saccharomyces cerevisiae* yeast having the advantage of multiplying in an acidic environment, with the optimum pH range between 4 and 4.5 [33].

Maintaining cytoplasmic pH is essential for yeast survival. The literature generally reports pH values between 2.8 and 8, with 4.5 and 6.5 being the ideal pH range [33,34].

1.4. Scope and Outline

Given this background, this research article aims to valorize, for the first time, tomato waste (skins, seeds, and peels) generated by the "Fouggara" tomato cannery in Reggane, Adrar, in South-Western Algeria, under Saharan climatic conditions, as a substrate for bioethanol production. The experimental study involves the coupled thermal and chemical pre-treatment of this substrate to facilitate alcoholic fermentation at laboratory scale. This paper provides a detailed characterization and experimental data. Since tomato waste is an abundant biowaste in many areas, the information from this paper enables the evaluation of the feasibility of similar processes by many stakeholders, as well as the validation of integrated process models.

The paper is organized into 3 other sections; Materials and Methods (section 2) providing detailed descriptions of the substrate preparation and the experimental equipment, Results and Discussion (Section 3) presenting the experimental findings obtained on the specific feedstock and a comparison with commercial references, and, finally, Conclusions (section 4).

2. Materials and Methods

2.1. The Substrate

The substrate under investigation consists of waste collected from the "Fouggara" tomato cannery (briefly referred to as TCW), located in Reggane, Southwest of the city of Adrar, in the Southwestern region of Algeria **(Figure 1a)**. This substrate is composed of peels, seeds, and skins **(Figure 1b)**. To prevent aerobic degradation of the substrate and microbial contamination, a drying process is carried out immediately after collection using an indirect solar dryer **(Figure 1c)**. The measured moisture content after drying is < 7%. Subsequently, the dried TCW is crushed using a Janke and Kunkel micro-mill to obtain particles smaller than 500 μm.

 doi:10.20944/preprints202412.1268.v1

a b c

Figure 1. Sampling of collected tomato waste (a), Crushed TCW (b), Indirect solar dryer (c).

The microorganism employed for the AF is the dry industrial baker's yeast, *Saccharomyces cerevisiae* **(Figure 2**) [35].

Figure 2. *Saccharomyces cerevisiae* yeast at natural scale (left) and under a photon microscope with 100X magnification (right).

2.2. Experimental Methodology for Alcoholic Fermentation

2.2.1. Samples Preparation

For the production of bio-alcohol through AF, 1-liter glass flask fermenters with 700 mL effective volume are employed and sealed airtight. These fermenters have 2 outlets: one for sampling and the other connected to a flask for the recovery of the produced gas, enabling the measurement of the carbon dioxide (CO_2) released. The movement of CO_2 bubbles released contributes to stirring the mixture, thus promoting AF. The reactors are incubated in a temperature-controlled water bath at 30 ± 1 °C [36].

TCW is mixed with water, in 4 different concentrations, namely 50, 75, 100, and 150 g/L. These mixes are tested in glass bioreactors to determine the optimal TCW concentration resulting in the best bioethanol production, see **Figure 3**.

 doi:10.20944/preprints202412.1268.v1

Figure 3. Bioreactors used for alcoholic fermentation.

2.2.2. Substrate Pretreatment

For the pretreatment of TCW, 2 methods are used:

- Steam explosion: to initiate the experiment, the 4 bioreactors are filled with 35, 52.5, 70, and 105 g of substrate and topped up with tap water to reach a total volume of 700 mL. The mixture undergoes thermal pretreatment in an autoclave at 140 °C and 2.5 bar for 40 minutes with 3 depressurization cycles to ensure the effective cracking of lignocellulosic bonds.
- Acid hydrolysis: following steam explosion, concentrated sulfuric acid (H_2SO_4) is added to the bioreactors in an amount equivalent to 5% of the working volume (3.5 mL in 700 mL). The treated bioreactors are then reintroduced into the autoclave for 50 minutes at 90 °C to break polysaccharide bonds and produce monomers [37]. Subsequently, to eliminate phenolic compounds that inhibit microbial activity during fermentation, calcium hydroxide ($Ca(OH)_2$) is added.

2.2.3. pH Adjustment

To ensure yeast proliferation and inhibit bacterial growth, the pH of the TCW should be maintained within the range of 4.3–4.7. To adjust the pH, some concentrated sulfuric acid was added.

2.3. Alcoholic Fermentation and Distillation

Sugar is necessary to reactivate the yeast, therefore 8.4 g of sugar are mixed with 0.7 grams of *Saccharomyces cerevisiae* yeast. Distilled water is then added to make up the volume to 100 mL. This solution is incubated in a preheated oven for 30 minutes at 30 °C.

The reactivated yeast is added to the previously adjusted bioreactors. They are incubated under anaerobic conditions in a water bath at 30 °C for 72 hours. All digesters are processed in triplicate and the results have been shown as means.

Using a 1-liter distillation column system, the fermented TCW is distilled to extract bioethanol. This system is heated using a round-bottom flask heater to the boiling temperature of ethanol at 78 °C [38,39]. The temperature is controlled at the top of the column. Vapors are taken to reflux to increase the ethanol concentration, and then they are subsequently condensed using a condenser, and the produced ethanol is collected in a glass flask.

2.4. Measurements: Targets and Equipment

AF is used to upgrade TCW for the bioethanol production process. Parameters such as ash content, protein, pH, reducing sugars, density, refractive index, Brix degree, and alcohol content during the 72 hours of AF must be monitored:

- Ash: ash content represents the total mass of mineral salts present in a fruit is calculated according to the American Society for Testing and Materials' (ASTM) standard methodology ASTM-E-1755-01 (2020) (Oxidation at 575± 25 °C standard test method for ash in biomass) [40].

 doi:10.20944/preprints202412.1268.v1

- Proteins: to assess the protein content, the total nitrogen content is determined. Nitrogen is essential for protein synthesis, which plays a role in transporting sugars into the cell during AF, where they are converted into bioethanol. The protein content is determined with the Kjeldahl method, which measures total nitrogen, essential for assessing protein content during fermentation processes [41].
- pH: it is determined with a pH meter (Mettler-Toledo AG, Analytical CH-8603 Schwerzenbach, Switzerland).
- Reducing sugars: the reducing sugars are measured as prescribed in the method of Dubois and Cool (1956) [42]
- Density Density measurements are performed at 20 °C by laboratory density meter based on oscillation (OIV-MA-AS2-01A:R2012)
- CO_2: the volume of CO_2 generated is measured using the liquid displacement method
- Refractive index: it is a dimensionless quantity characteristic of a medium, describing the behavior of light in that medium. An Abbé refractometer (Standard NF ISO 2173: 2003) is used to measure refractive index.
- Brix degree/soluble solids: the total soluble solids, expressed as °Brix %, are measured from homogeneous filtered fruit juice using an Abbé refractometer. (Standard NF ISO 2173: 2003).
- Alcohol content: an alcoholmeter with a range of 0-100° (standard OIV-MA-AS312-01A: R2009) is used to measure alcoholic content.
- Yeast evolution: yeast proliferation under a microscope over the course of 72 hours of fermentation is measured. The evolution of the yeast is monitored by hemocytometric counting (Malassez Cell) of the microbial population under an optical microscope type (OPTIKA B-350, G: 100×).
- For sampling from the flasks, as shown in Figure 3, there are 2 holes, one for CO_2 removal and the other for sampling. The sample volume (after mixing and homogenization) does not exceed 3 mL, which has no influence on the total reactor volume.
- Finally, bioethanol is characterized using Agilent Cary 660 Fourier Transform Infrared (FT-IR) infrared spectrometer. Chromatographic analyses were carried out on an electronically pressure-controlled PerkinElmer Gas Chromatography (GC) (Clarus 500 series). The instrument is controlled by a "Total Chrom Tutorial, version 6.3" computer system.

3. Results and Discussion

The results of alcoholic fermentaion are presented here, from the fermentation process to bioethanol extraction.

3.1. Fermentation Parameters

Figure 4 portrays the trends of measured parameters during the 72-h test on TCW. All parameters are discussed in the following sub-paragraphs.

3.1.1. Ash Content

The value found for the TCW is 3.57% of Total Solids (TS), which is close to that found by Sarada et al., [43], with 4.55%. This high value explains the richness of tomato waste in mineral elements. According to **Figure 4a**, the ash values of TCW during AF decreased from 3.57% to 2.41%. This reduction in ash content during AF is the result of the mineral salts consumed by the yeast during the sugar degradation process.

 doi:10.20944/preprints202412.1268.v1

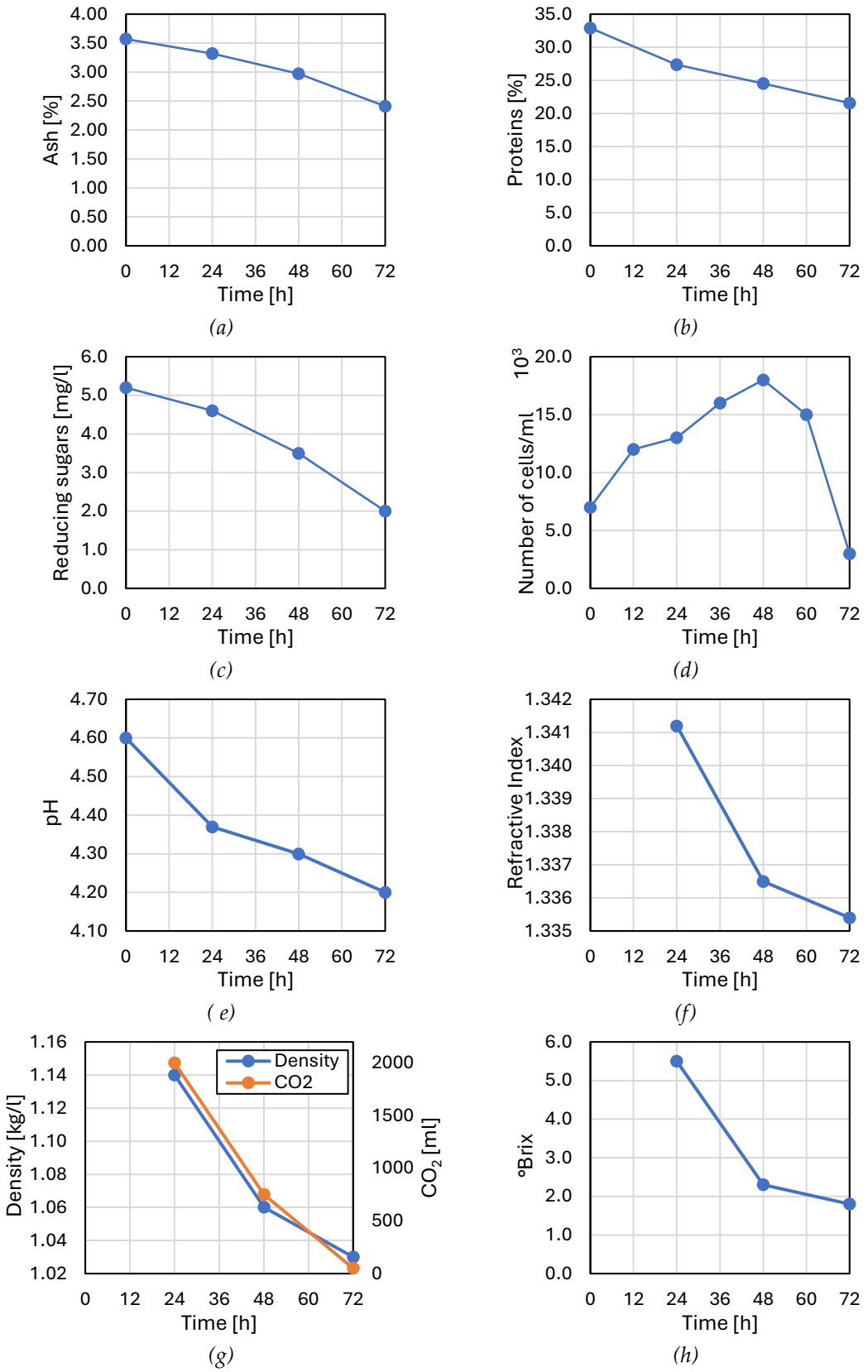

Figure 4. Evolution of the substrate over 72-h fermentation: (a) ash, (b) proteins, (c) reducing sugars, (d) cells count, (e) PH, (f) refractive index, (g) density and CO_2, (h) Brix index.

3.1.2. Proteins

The total nitrogen content in TCW is 5.5%, and the protein content is estimated at 34.4%. These values are higher than those found by Persia et al. [44] and Seddiki [45]. In the study by Persia et al.

 doi:10.20944/preprints202412.1268.v1

(2003), the nutritional profile of dried tomato seeds is evaluated, highlighting their potential as a valuable feed ingredient. The research focuses on the chemical composition, including protein, fat, and fiber content, and assesses the seeds' overall nutritional value for poultry diets. Similarly, the study carried out by Seddiki focuses on the valorization of tomato by-products, emphasizing their potential in enhancing food security and sustainability. The research highlights innovative methods for utilizing these by-products, which are often discarded, to create value-added products (protein 24.5%).

This protein content will be used as a source of nitrogenous materials in organic or inorganic form, which is essential during yeast multiplication to meet the nitrogen requirements for the formation of amino acids, nucleotides, and certain vitamins [46]. Yeasts are capable of utilizing inorganic nitrogen in the form of ammonium ions (chloride, nitrate, phosphate, and notably sulfate, which is the ideal compound as it provides the sulfur necessary for the synthesis of certain amino acids) [47].

According to **Figure 4b**, it can be observed that the protein content decreases during AF. This decrease is likely a result of yeast assimilating the proteins to complete the AF.

3.1.3. Reducing Sugar Content

The levels of reducing sugars are shown in Figure 4c. The results obtained show a significant decrease during the AF process, where the sugar content drops from 5.24 to 2.02 mg/L at the end of the AF. This result agrees with the reported findings by [48]. This decrease is likely due to biomass production and sugar assimilation. Furthermore, yeast uses sugars to generate energy and support cell growth. They are capable of converting glucose into ethanol and CO_2, with the coproduction of glycerol, esters, and certain acids [48–50].

3.1.4. Cell Count

Yeast proliferation under a microscope over the course of 72 hours of fermentation was monitored, and the results are shown in **Figure 4d**. The curve indicates that the stages of *Saccharomyces cerevisiae* proliferation over time are typical of those of all microorganisms. This shows a conventional growth curve with a latent phase, an increasing phase, an exponential phase, a stationary phase, and a declining phase, during which the high ethanol content inhibits yeast proliferation [51]. To confirm these results, a comparison is made with this curve to the MONOD model [52], a similarity in the following notable 5 distinct phases was observed:

- Phase 1 (0-12h): Latency phase during which cells synthesize the enzymes needed to metabolize the substrate present. Cell reproduction is nearly non-existent during this phase.
- Phase 2 (12-24h): Acceleration phase where actual growth initiation occurs.
- Phase 3 (24-36h): Exponential growth phase with cell reproduction reaching its maximum at a constant rate. This is due to the richness of TCW in nutrients (mineral compounds, vitamins, carbon, and nitrogen compounds), providing an energy source [53]. Additionally, the nitrogen source is significant because it contributes to the structure of nucleic acids and proteins, important constituents of Saccharomyces cerevisiae cells [54].
- Phase 4 (36-60h): Stationary phase with cell concentration remaining relatively constant, indicating cessation of cell reproduction. This is a sign of exhaustion of nutritional sources, namely glucose, and accumulation of inhibitory products resulting from microbial metabolism, where ethanol concentration increases in the medium (Figure 12). Growth rate, metabolic activity, cell viability, and yeast production capacity are all declining. Canetta et al., [55] confirmed these phenomena in their work, especially in *Saccharomyces cerevisiae*.
- Phase 5 (60-72h): Decline phase with a decrease in cell mass due to autolysis and a negative growth rate. This is attributed to ethanol causing stress in yeast and becoming toxic at high concentrations. These findings have been validated by several researchers, particularly in Saccharomyces cerevisiae [54–57].

3.1.5. pH Evolution

Figure 4e shows a decrease in the pH of TCW. This pH decrease is likely due to sugar consumption and bioethanol production, as well as the acids and alcohols metabolized by microorganisms (yeasts) present in the reaction mixture, such as fatty acids, especially octanoic and

 doi:10.20944/preprints202412.1268.v1

decanoic acids. Furthermore, this decrease in intracellular pH can be due to either proton influx or the accumulation of reaction intermediates such as acetic acid and glycerol [58]. Additionally, some of the CO_2 produced dissolves in the mixture, contributing to the lowering of pH. This result shows that yeast is able to tolerate numerous organic acids up to pH 4.5. Nevertheless, lactic, citric, and acetic acids significantly inhibit yeast growth, as well as sorbic and propionic acids to a greater extent [53]. There are other factors that can affect pH, as described by Jones et al., [59], in which ethanol stress leads to a decrease in cytoplasmic pH, resulting in yeast death.

3.1.6. Refractive Index

The refractive index values decrease from 1.3412 to 1.3354 during fermentation **(Figure 4f)**. This decrease can be explained by the increase in the speed of light during fermentation, which is directly related to the decrease in density [36].

3.1.7. Density

According to the results presented in **Figure 4g**, a significant decrease in density was observed. Density values are recorded after every 24 hours, showing a mildly decreasing trend from 1.14 to 1.03 kg/L. During the fermentation process, the loss of mass in the form of CO_2 and the conversion of glucose into alcohol account for this fall in density [60].

3.1.8. Soluble Solids Content

According to the presented results in **Figure 4h**, a decrease in soluble solids content is observed, primarily due to the reduction in sucrose concentration in the fermented solution, expressed in degrees Brix (°Brix). This indicator decreases from 5.5 °Brix to 1.8 °Brix.

3.1.9. Alcohol Degree

At the beginning of fermentation, the alcohol content is zero. However, after 24 hours, a value of 2° is recorded. Alcohol production becomes significant during the last 48 hours, increasing from 6° to 12° at the end of fermentation (after 72 hours). Similar observations were reported by El Ogaïdi [61], who estimated a fermentation time between 36 and 72 hours. **Table 1** illustrates the changes in the appearance of the fermented mixture over time, together with the increase of the alcoholic degree. The results obtained have shown that TCW produces ethanol through AF, with a final alcohol content of 12°.

Table 1. Results of the alcoholic fermentation of TCW.

Duration	Alcoholic degree	Comments
0 h	0°	Mixture color: Brown
24 h	2°	Appearance of 2 layers
48 h	6°	Precipitate color: Dark brown
72 h	12°	The supernatant becomes increasingly clear (yellowish). The residue turns brown.

3.2. Characterization of the "Bioethanol" Fermentation Product

The results of the physicochemical properties of the bioethanol produced from TCW are presented in **Table 2**, in comparison of bioethanol produced from another feedstock (potato waste [62]) and the commercial reference.

Table 2. Characteristics of the bioethanol produced in the laboratory from TCW.

Parameter	Bioethanol from TCW	Bioethanol from Potato Waste	Commercial ethanol
Density	0.886 kg/L	0.662 kg/L	0.789 kg/L

 doi:10.20944/preprints202412.1268.v1

Refractive index	1.3628	1.395	1.3594
Alcohol content after 1st distillation	12°	-	-
Alcohol content after rectification	54°	-	-

The characteristics of the laboratory-produced bioethanol are similar to those of commercial ethanol. Comparatively, the density of the ethanol solution produced from TCW is 0.886 kg/L, whereas that of commercial ethanol is 0.789 kg/L. Laboratory-produced bioethanol **(Figure 5)** exhibits the following characteristics: volatility, flammability, clarity, and a pungent odor.

Figure 5. Characteristics of ethanol produced from TCW: flame (left), colorless liquid (right).

3.2.1. Characterization of Bioethanol Obtained by IR Spectrophotometry

Figure 6 represents the vibrations of the bands obtained by infrared spectrophotometry of the bioethanol produced by AF of TCW. The infrared spectrum of the final product obtained through fermentation after distillation is characterized by the vibrations of the following bands: 2977 cm^{-1} and 3341 cm^{-1}, corresponding respectively to the C-H groups of an alkane and the specific O-H group of an alcohol. A comparison between the infrared spectra of ethanol produced from TCW and that of commercial ethanol shows agreement, with vibration bands of different groups appearing at the same frequency. Boulal et al., [23] obtained identical findings.

A comparison between the GC spectra of pure ethanol and the bioethanol produced from tomato waste **(Figure 7)** shows that the peaks of the ethanol molecule in the GC spectrum are visible at a retention time of 0.9. The second peak appearing is likely due to impurities in the bioethanol produced. This observation aligns with the findings of Boulal et al., [23].

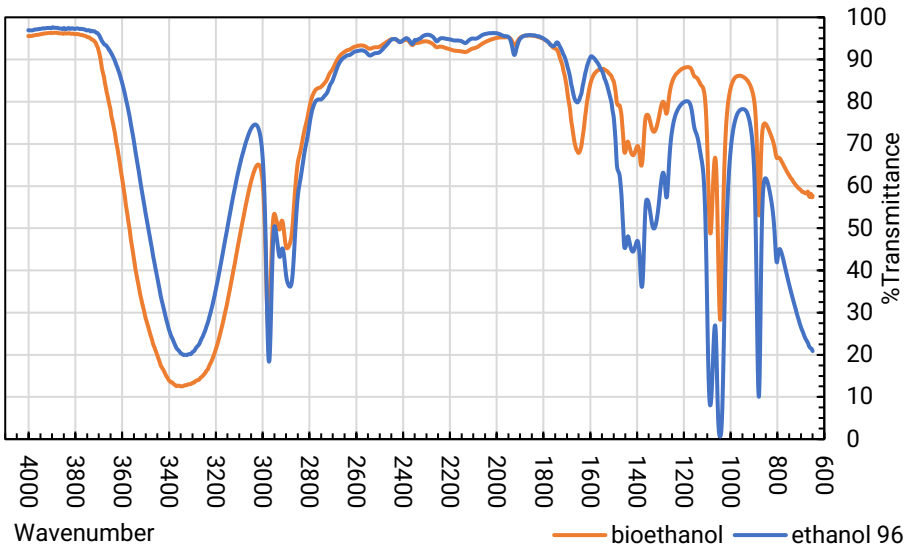

Figure 6. IR spectrum: TCW bioethanol in comparison with commercial ethanol.

 doi:10.20944/preprints202412.1268.v1

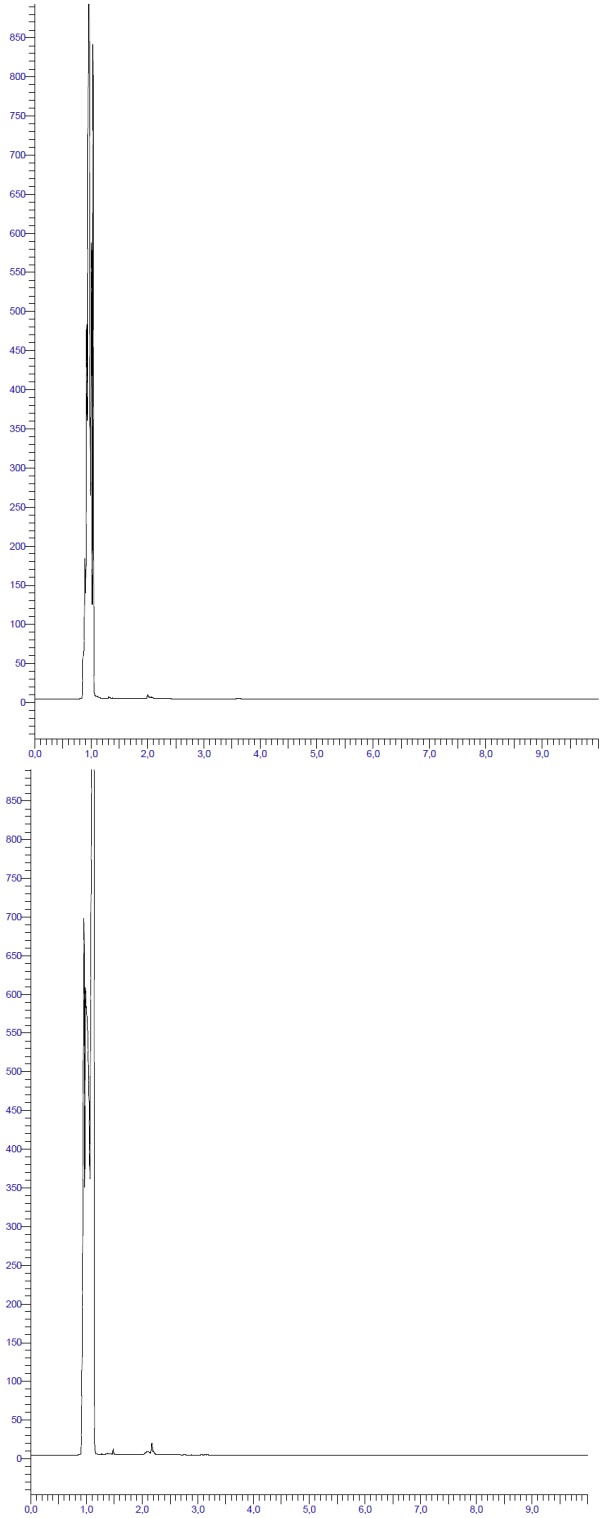

Figure 7. Chromatogram of commercial ethanol (left), Chromatogram of TCW-derived bioethanol (right).

4. Conclusions

In this study, the solid residue that remains after processing tomatoes to create tomato juice, paste, sauce, puree, or ketchup commonly known as tomato canning waste was used for the first time as a feedstock for bioethanol production by alcoholic fermentation using thermochemical pretreatment. TCW was obtained from the "Fouggara" tomato canning factory in Reggane/Adrar, South-West Algeria.

The obtained results are very promising and show that the bioethanol produced is characterized by an alcohol content of 12° over a process period of 72 hours with a density of 0.886 kg/L, close to

 doi:10.20944/preprints202412.1268.v1

that of commercial ethanol at 0.789 kg/L. In addition, its characteristics are very close to those of commercial ethanol. Several parameters were involved to obtain these results, namely the acid pH of the TCW, which favors the growth of the *Saccharomyces cerevisiae* yeast used in the fermentation process. The evolution of this yeast over time follows a curve typical of the MONOD model, with phases of latency, acceleration, exponential growth, a stationary phase, and a decline phase. During the stationary and decline phases, the high ethanol content inhibits yeast proliferation. The density also decreases from 1.14 to 1.03 kg/L due to the significant release of carbon dioxide (CO_2) during AF, resulting from the transformation of glucose into alcohol and the loss of mass in the form of CO_2. Reducing sugars dropped from 5.24 to 2.02 mg/L at the end of fermentation due to the biomass production and sugar assimilation. Since the results are very interesting, further research to this scope could improve the quantity and quality of the produced bioethanol to reach sustainable development goals.

Nomeclature

AD Anaerobic Digestion; AF Alcoholic Fermentation; CH_4 Methane; CO_2 Carbon Dioxide; FT-IR Fourier Transform Infrared; GC Gas Chromatography; GHG Green House Gas; IR Infrared; TCW Tomato Canning Waste; TS Total Solids.

Author Contributions: Conceptualization, K.K.; methodology, M.D.; software, F.S.; validation, A.E.A.; formal analysis, A.T.; investigation, M.K.; resources, M.D; data curation, F.S.; writing—original draft preparation, A.A.; writing—review and editing, A.B., A.E.A.; visualization, K.K and A.B.; supervision, A.E.A.; project administration, S.K.; funding acquisition, S.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research work was funded by the General Directorate of Scientific Research and Technological Development (DGRSDT) Algeria, Project 177/2017 (Bio-fuel production from Saharan biomass in a continuous system using solar energy).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: This research work was funded by the General Directorate of Scientific Research and Technological Development (DGRSDT) Algeria.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Bibra, M.; Samanta, D.; Sharma, N.K.; Singh, G.; Johnson, G.R.; Sani, R.K. Food Waste to Bioethanol: Opportunities and Challenges. Fermentation 2022, 9, 8.
2. Sikiru, S.; Abioye, K.J.; Adedayo, H.B.; Adebukola, S.Y.; Soleimani, H.; Anar, M. Technology Projection in Biofuel Production Using Agricultural Waste Materials as a Source of Energy Sustainability: A Comprehensive Review. Renew. Sustain. Energy Rev. 2024, 200, 114535.
3. Branthôme, F.-X. Worldwide (Total Fresh) Tomato Production in 2021 - Tomato News.
4. Mimouni, C. Plans de Développement Agricole En Algérie: Etat Des Lieux et Leur Impact Sur La Production Agricole de 2000 à 2019. Rev. Financ. marchés 2023, 10, 121–142.
5. Generale, R.; Terres, D.E.S. République Algérienne Démocratique et Populaire Ministère de l ' Agriculture et Du Développement Rural Direction Des Statistiques Agricoles et Des Systèmes d ' Information SUPERFICIES ET PRODUCTIONS JUILLET 2021. 2021, 1–87.
6. Shrestha, N.; Fogg, A.; Wilder, J.; Franco, D.; Komisar, S.; Gadhamshetty, V. Electricity Generation from Defective Tomatoes. Bioelectrochemistry 2016, 112, 67–76.
7. Del Valle, M.; Cámara, M.; Torija, M. Chemical Characterization of Tomato Pomace. J. Sci. Food Agric. 2006, 86, 1232–1236.
8. Garcia-Marti, M.; Simal-Gandara, J. Chemical and Biological Valorization of Tomato Waste. 2023.
9. Christofi, A.; Fella, P.; Agapiou, A.; Barampouti, E.M.; Mai, S.; Moustakas, K.; Loizidou, M. The Impact of Drying and Storage on the Characteristics of Two-Phase Olive Pomace. Sustainability 2024, 16, 1116.
10. Pemas, S.; Gkiliopoulos, D.; Samiotaki, C.; Bikiaris, D.N.; Terzopoulou, Z.; Pechlivani, E.M. Valorization of Tomato Agricultural Waste for 3D-Printed Polymer Composites Based on Poly (Lactic Acid). Polymers (Basel). 2024, 16, 1536.
11. Drescher, A.; Schwingshackl, L.; Kienberger, M. Identification of Molecules from Tomato Plant Residues Using Sustainable Green Chemicals. 2024.

12. Farinon, B.; Felli, M.; Sulli, M.; Diretto, G.; Savatin, D. V; Mazzucato, A.; Merendino, N.; Costantini, L. Tomato Pomace Food Waste from Different Variants as a High Antioxidant Potential Resource. Food Chem. 2024, 452, 139509.
13. Rashwan, M.; Abd El-Razzak, H.; Alkoaik, F.; Ibrahim, M.; Fulleros, R. Influence of Tomato Waste Composts on Dry Matter and Heavy Metals Content in Cucumber and Summer Squash Seedlings. J. Air Waste Manage. Assoc. 2023, 73, 695–704.
14. Chen, T.; Wang, Q.; Wang, Y.; Dou, Z.; Yu, X.; Feng, H.; Wang, M.; Zhang, Y.; Yin, J. Using Fresh Vegetable Waste from Chinese Traditional Wet Markets as Animal Feed: Material Feasibility and Utilization Potential. Sci. Total Environ. 2023, 902, 166105.
15. Heuzé, V.; Tran, G.; Hassoun, P.; Bastianelli, D.; Lebas, F. Tomato Pomace, Tomato Skins and Tomato Seed. 2015.
16. Arroussi, A.; Tahri, A.; Kalloum, S.; Abdelli, N.; Bouziane, N. Valorization Of Lignocellulosic Material From Date Palm Waste (Phoenix Dactylefera L.) Elhmira Cultivar By Composting As Organic Fertilizer. UPB Sci Bull, Ser B 2022, 84, 59–72.
17. Tawantum, J.; Muensit, N.; Sathapondecha, P.; Mamimin, C. Valorization of Agro-Industrial Solid Waste by Two-Stage Anaerobic Digestion for Biohythane Production. ASEAN J. Sci. Technol. Reports 2024, 27, e253769–e253769.
18. Arroussi, A.; Tahri, A.; Nehar, O.K.; Soulimani, A.E.-A.; Dahou, M.E.-A. VALORIZATION OF DATE WASTE (HMIRA CULTIVAR) FOR THE PRODUCTION OF BIOALCOHOL AND BIOGAS. Curr. Trends Nat. Sci. 2023, 12, 78–91.
19. Durmuş, M.; Kızılkaya, R. The Effect of Tomato Waste Compost on Yield of Tomato and Some Biological Properties of Soil. Agronomy 2022, 12, 1253.
20. Mekunye, F.; Makinde, P. Production of Biofuels from Agricultural Waste. Asian J. Agric. Hortic. Res. 2024, 11, 37–49.
21. Rola, K.; Gruber, S.; Goričanec, D.; Urbancl, D. Waste Lignocellulosic Biomass as a Source for Bioethanol Production. Sustain. Chem. 2024, 5, 1–12.
22. Yerizam, M.; Jannah, A.M.; Aprianti, N.; Yandriani, Y.; Rendana, M.; Ernas, A.Q.; Tamba, J.L. Bioethanol Production from Coconut Husk Using DES-NADES Pretreatment and Enzymatic Hydrolysis Method. Comptes Rendus. Chim. 2023, 26, 1–10.
23. Vieira, R.C.; De Farias Silva, C.E.; da Silva, L.O.M.; Almeida, R.M.R.G.; de Oliveira Carvalho, F.; dos Santos Silva, M.C. Kinetic Modelling of Ethanolic Fermented Tomato Must (Lycopersicon Esculentum Mill) in Batch System: Influence of Sugar Content in the Chaptalization Step and Inoculum Concentration. React. Kinet. Mech. Catal. 2020, 130, 837–862.
24. Vieira, R.C. Estudo Da Modelagem Cinética Da Fermentação Alcoólica Em Batelada de Polpa de Tomate (Lycopersicon Esculentum Mill). 2020.
25. Osei, J.A. Feasibility of Bioethanol Production from Rotten Tomatoes (Solanum Lycopersicum) Using Saccharomyces Cerevisiae. Waste Technol. 8, 34–38.
26. Many, J.N.; Radhika, B.; Ganesan, T. Study on Tomato Wine Production and Optimization. J. Environ. Sci. Toxicol. Food Technol. 2014, 97–100.
27. Deng, K.; Li, E. Study on Preparation of Low Alcoholic Wine from Tomato. In Proceedings of the Proceedings of the 2012 International Conference on Applied Biotechnology (ICAB 2012) Volume 2; Springer, 2014; pp. 931–941.
28. Yuan, J.; Zhang, H.; Zeng, C.; Song, J.; Mu, Y.; Kang, S. Impact of Fermentation Conditions on Physicochemical Properties, Antioxidant Activity, and Sensory Properties of Apple–Tomato Pulp. Molecules 2023, 28, 4363.
29. Jang, S.-Y.; Woo, S.-M.; Jo, Y.-J.; Kim, O.-M.; Kim, I.-H.; Jeong, Y.-J. Quality Characteristics of Tomato Wine on Fermentation Conditions. J. Korean Soc. Food Sci. Nutr. 2010, 39, 443–448.
30. Watanabe, M.; Tamura, K.; Magbanua, J.P.; Takano, K.; Kitamoto, K.; Kitagaki, H.; Akao, T.; Shimoi, H. Elevated Expression of Genes under the Control of Stress Response Element (STRE) and Msn2p in an Ethanol-Tolerance Sake Yeast Kyokai No. 11. J. Biosci. Bioeng. 2007, 104, 163–170.
31. Bocquet, J. Généralités Sur Les Microorganismes. Tec Doc Lavoisier, Paris 1982, 11.
32. Pishgar, R.; Najafpour, G.; Neya, B.N.; Mousavi, N.; Bakhshi, Z. Anaerobic Biodegradation of Phenol: Comparative Study of Free and Immobilized Growth. Iran. J. Energy Environ. 2011, 2, 348–355.
33. Revuz, B. Microbiologie et Industrie Alimentaire (Culture de La Levure Sur Mêlasse).(Ed) Lavoisier 1979.
34. Bourgeois, C.M.; Larpent, J.P. Microbiologie Alimentaire: Aléments Fermentés et Fermentations Alimentaires; Lavoisier, 1996; ISBN 2743000805.
35. El Hadj, M.D.O.; Dan-Badjo, A.T.; Halouane, F.; Doumandji, S. Toxicité Comparée Des Extraits de Trois Plantes Acridifuges Sur Les Larves Du Cinquième Stade et Sur Les Adultes de Schistocerca Gregaria Forskål, 1775 (Orthoptera-Cyrtacanthacridinae). Sci. Chang. planétaires/Sécheresse 2006, 17, 407–414.

36. Boulal, A.; Khelafi, M.; Kaidi, K. Procédés Expérimentales Sur La Production de Bioéthanol de Deuxième Génération à Partir Des Sous-Produits Des Palmiers Dattiers. Rev. des Energies Renouvelables SIENR 2018, 18, 63–69.
37. Didderen, I.; Destain, J.; Thonart, P. Le Bioéthanol de Seconde Génération: La Production d'éthanol à Partir de Biomasse Lignocellulosique; Presses agronomiques de Gembloux, 2008; ISBN 2870160852.
38. Kraiem, N.; Lajili, M.; Limousy, L.; Said, R.; Jeguirim, M. Energy Recovery from Tunisian Agri-Food Wastes: Evaluation of Combustion Performance and Emissions Characteristics of Green Pellets Prepared from Tomato Residues and Grape Marc. Energy 2016, 107, 409–418.
39. Kaidi, F.; Touzi, A. Production de Bioalcool à Partir Des Déchets de Dattes. Rev. des Energies Renouvelables, NS Biomasse Prod. Valoris. 2001, 75–78.
40. Amellal nee Chibane, H. Aptitudes Technologique de Quelques Variétés Communes de Dattes: Formulation d'un Yaourt Naturellement Sucré et Aromatisé 2008.
41. Aguirre, J. The Kjeldahl Method. In The Kjeldahl Method: 140 Years; Springer, 2023; pp. 53–78.
42. DuBois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A. t; Smith, F. Colorimetric Method for Determination of Sugars and Related Substances. Anal. Chem. 1956, 28, 350–356.
43. Sarada, R.; Nand, K. Start-up Anaerobic Digestion of Tomato-Processing Wastes for Methane Generation. Biol. wastes 1989, 30, 231–237.
44. Persia, M.E.; Parsons, C.M.; Schang, M.; Azcona, J. Nutritional Evaluation of Dried Tomato Seeds. Poult. Sci. 2003, 82, 141–146.
45. SEDDIKI, D. Valorisation Des Sous-Produits de Tomates 2021.
46. Booth, C. Methods in Microbiology; Academic press, 1971; ISBN 0080860303.
47. Leveau, J.-Y.; Bouix, M. Microbiologie Industrielle: Les Micro-Organismes d'intérêt Industriel. 1993.
48. Jeguirim, M.; Jellali, S.; Khiari, B. Foreword: Recent Advances in Sustainable Biomass Valorization. Comptes Rendus. Chim. 2022, 25, 1–5.
49. Leyral, G.; Vierling, E.; Figarella, J.; Zonsain, F. Microbiologie et Toxicologie Des Aliments: Hygiène et Sécurité Alimentaires; Doin, 1997; ISBN 2704007055.
50. Jellali, S.; Hamdi, N.; Riahi, K.; Hamdi, H.; Jeguirim, M. Environmental Applications of Tomato Processing By-Products. In Tomato Processing by-Products; Elsevier, 2022; pp. 231–284.
51. Jiang, Y.; Liang, C.; Zhao, W.; Chen, T.; Yu, B.; Hou, A.; Zhu, J.; Zhang, T.; Liu, Q.; Ying, H. Cell Cycle Progression Influences Biofilm Formation in Saccharomyces Cerevisiae 1308. Microbiol. Spectr. 2022, 10, e02765-21.
52. Vallino, J.J.; Hopkinson, C.S.; Hobbie, J.E. Modeling Bacterial Utilization of Dissolved Organic Matter: Optimization Replaces Monod Growth Kinetics. Limnol. Oceanogr. 1996, 41, 1591–1609.
53. Jeguirim, M.; Khiari, B. Biofuels Production. In Palm Trees and Fruits Residues; Elsevier, 2023; pp. 351–391.
54. Ammouche, A. Contribution a l'etude de La Concentration En Methanol et de La Nature de La Source Azotee Sur La Croissance de La Levure (Hansenula Polymorpha). 1979.
55. Canetta, E.; Adya, A.K.; Walker, G.M. Atomic Force Microscopic Study of the Effects of Ethanol on Yeast Cell Surface Morphology. FEMS Microbiol. Lett. 2006, 255, 308–315.
56. Aguilera, F.; Peinado, R.A.; Millan, C.; Ortega, J.M.; Mauricio, J.C. Relationship between Ethanol Tolerance, H+-ATPase Activity and the Lipid Composition of the Plasma Membrane in Different Wine Yeast Strains. Int. J. Food Microbiol. 2006, 110, 34–42.
57. Hirasawa, T.; Yoshikawa, K.; Nakakura, Y.; Nagahisa, K.; Furusawa, C.; Katakura, Y.; Shimizu, H.; Shioya, S. Identification of Target Genes Conferring Ethanol Stress Tolerance to Saccharomyces Cerevisiae Based on DNA Microarray Data Analysis. J. Biotechnol. 2007, 131, 34–44.
58. Birch, R.M.; Walker, G.M. Influence of Magnesium Ions on Heat Shock and Ethanol Stress Responses of Saccharomyces Cerevisiae. Enzyme Microb. Technol. 2000, 26, 678–687.
59. Jones, R.P.; Pamment, N.; Greenfield, P.F. Alcohol Fermentation by Yeasts-the Effect of Environmental and Other Variables. Process Biochem. Kingdom) 1981, 16.
60. Gaillard, J.L.; Leclerc, H.; Simonet, M. Microbiologie Générale. Ed. Doin, Paris 1995, 535.
61. El Ogaïdi, H.K.H. Dates and Confectionery Product. FAO, Rome 1987, 1–25.
62. Bouhadi, D.; Larbi, K.S.; Belkhodja, H.; Belmimoun, A.; Habib, A.; Semmache, A.; Ouahdi, M. Bioethanol Production from Potato Peel Waste Using Saccharomyces Cerevisiae. Acta Agric. Serbica 2023, 28.