Moroccan Journal of Agricultural Sciences
Vol 6 No 3 (2025): (September 2025), 193-207
Nutrition and Food Technology

Biotechnological valorization of citrus sorting rejects into bioethanol and acetic acid using indigenous microorganisms in Morocco

Kawtar FARIH — Biotechnology and Biotransformation Laboratory, Department of Food Science and Nutrition, Hassan II Institute of Agronomy and Veterinary Medicine, Rabat, Morocco

Alexander LUNGOWE — Biotechnology and Biotransformation Laboratory, Department of Food Science and Nutrition, Hassan II Institute of Agronomy and Veterinary Medicine, Rabat, Morocco.

Majid MOUNIR — Biotechnology and Biotransformation Laboratory, Department of Food Science and Nutrition, Hassan II Institute of Agronomy and Veterinary Medicine, Rabat, Morocco

The expanding citrus industry in Morocco generates large volumes of underutilized by-products and sorting rejects. Rich in fermentable sugars and bioactive compounds, these residues offer potential for biotechnological conversion. This study focused on valorizing citrus sorting rejects by isolating native microorganisms for bioethanol and acetic acid production. Juices from sorting rejects of four citrus varieties were used as fermentation substrates. Yeast and acetic acid bacteria were isolated from Moroccan sourdough and traditional vinegars, then screened for fermentation efficiency. Yeast strain L4 and acetic acid bacteria strain AV22 showed superior performance compared to a commercial *Saccharomyces cerevisiae* strain. L4 produced 4.03% (v/v) ethanol from Sanguinelli juice without sucrose, and 11.4% with added sucrose. The fermented must yielded 8.40 L of vinegar containing 5.56% (w/v) acetic acid in a 10 L bioreactor, and was successfully scaled up to 80 L of vinegar with 7.0% (w/v) acidity in a 500 L pilot-scale acetifier. Sensory evaluation by 51 untrained panelists confirmed the vinegar's high acceptability. This work demonstrates the potential of indigenous strains in converting citrus waste into value-added products, promoting sustainable waste management and circular bioeconomy practices. Future research will focus on process optimization, scaling up, and co-product recovery to improve economic feasibility.

Keywords: Yeast, acetic acid bacteria, bioethanol, acetic acid, citrus by-products, screening, fermentation, citrus vinegar, waste valorization

INTRODUCTION

Citrus fruits such as oranges, lemons, and grapefruits represent a significant component of Morocco's agricultural economy. Thanks to the country's favorable Mediterranean climate, fertile soils, and long-standing agronomic tradition, citrus cultivation has expanded considerably in recent decades. Morocco ranks among the top citrus producers in Africa, with oranges and small citrus fruits accounting for the majority of national production (MAPMDREF, 2020; FAO, 2021). This expansion, however, has been accompanied by an increase in citrus processing activities, generating large volumes of by-products such as peels, pulp, seeds, and sorting rejects. These residues, if left unexploited, can become major environmental liabilities due to their high organic

load and rapid biodegradability (Zema et al., 2018; Grigoraş, 2012).

In recent years, growing concerns over environmental sustainability and the need for circular economy strategies have spurred intense interest in the valorization of agricultural by-products. Citrus residues are particularly attractive in this context due to their chemical richness in fermentable sugars, organic acids, essential oils, flavonoids, and pectin (Albrigo et al., 2019; Berk, 2016). Biotechnological processes have emerged as promising approaches to transform these wastes into value-added products such as bioethanol and acetic acid, which are widely used as renewable fuels, food additives, and platform chemicals (Vasić et al., 2021; Choi et al., 2015). This dual benefit—waste mitigation and resource generation - makes citrus by-product and sorting rejects valorization a relevant model for sustainable agro-industrial development.

Among the biotechnological pathways explored, microbial fermentation plays a central role. Bioethanol production typically involves enzymatic hydrolysis followed by alcoholic fermentation, often using Saccharomyces cerevisiae or thermotolerant yeasts such as Kluyveromyces marxianus (Taherzadeh and Karimi, 2007; Hasan and Sardar, 2021). However, citrus peels contain d-limonene, a monoterpene known to inhibit microbial activity, thereby complicating the fermentation process (Choi et al., 2015). Effective pretreatment strategies - such as enzymatic hydrolysis, steam explosion, or solvent extraction - are thus required to enhance fermentability while minimizing microbial inhibition (Klein-Marcuschamer et al., 2012; John et al., 2017).

In parallel, acetic acid production via vinegar fermentation also constitutes an attractive valorization route. It involves a two-stage bioprocess: an initial anaerobic alcoholic fermentation followed by an aerobic oxidation of ethanol into acetic acid, typically using Acetobacter or Komagataeibacter strains (Lynch et al., 2019; Mounir et al., 2016). These acetic acid bacteria (AAB) possess the ability to oxidize a variety of carbon sources, including ethanol and glucose, into acetic acid and other organic acids such as gluconic or galactonic acids (Deppenmeier and Ehrenreich, 2008; Gomes et al., 2018). Vinegar produced from citrus juices or by-products has shown not only high sensory quality but also enhanced functional properties due to the retention of bioactive compounds such as flavonoids, limonoids, and ascorbic acid (María Luzón-Quintana et al., 2021).

In addition to liquid biofuels and food-grade acids, citrus residues can also be converted into other valuable bioproducts. These include dietary fibers, pectin-based edible films, antioxidant extracts, animal feed, and biofertilizers (Grigoraş, 2012; Ademosun et al., 2016; Suhag et al., 2020). The citrus pulp and peels, rich in soluble sugars and fibers, also serve as excellent substrates for the growth of microbial biomass such as Candida utilis, which can be enriched with micronutrients like selenium (Stabnikova et al., 2005). The incorporation of citrus by-products into the food industry - as functional ingredients in beverages, bakery goods, and dairy products - has also gained momentum due to consumer interest in sustainable and health-enhancing foods (Ademosun, 2022; Mahmoud et al., 2016).

Given the nutritional potential, chemical richness, and fermentability of citrus by-products, their transformation through microbial fermentation offers a compelling route for sustainable valorization. However, to unlock this potential, careful microbial screening, substrate optimization, and process design are required. This study aims to screen, isolate, and evaluate microbial strains capable of efficiently converting citrus by-products into bioethanol and vinegar under laboratory conditions. The ultimate objective is to assess the feasibility of establishing an integrated bioprocessing approach that links citrus waste management with bio-based production chains, thereby reducing environmental burden and supporting bioeconomy development in citrus-producing regions such as Morocco.

This study aims to isolate and screen native yeast and AAB strains capable of converting citrus juice and by-products into bioethanol and vinegar. The approach emphasizes low-cost substrates, circular economy principles, and the integration of indigenous microbial biodiversity. Ultimately, it offers a dual benefit: reducing agro-industrial waste and generating eco-friendly, value-added

products suitable for food and bioenergy applications.

MATERIALS AND METHODS

Raw Materials and Sample Preparation

A total of four citrus cultivars - Maroc Late, Sanguinelli, Pomelo, and Nadorcott - were harvested from two agroecological zones in Morocco: Sidi Kacem and Sidi Slimane. Harvests were conducted at different physiological maturity stages to reflect variations in biochemical composition. Fruits were washed under running tap water to remove soil and residues, and then air-dried at ambient temperature.

After initial washing, fruits were weighed individually and cut longitudinally. Juice extraction was performed using a Look'In Lamacom® juicer. The juice was filtered through a stainless-steel sieve (0.2 mm^2 pore size) to remove pulp residues and seeds. Due to segment detachment during extraction, Nadorcott samples required an additional homogenization step using a laboratory blender, followed by filtration through cheesecloth. All juices were aliquoted into sterilized 5 L polyethylene bottles and stored at –20 °C. Pulp and peels were kept refrigerated at 4 °C.

Morphological and Physical Characterization of Citrus Fruits

Fruit morphology (diameter, shape, peel color, segment number, seed presence, peel thickness, and pulp characteristics) was documented using visual and tactile analysis. Fruit diameter was measured with a precision ruler, and representative cross-sections were photographed.

Physical parameters of juice included pH (pH-meter, Mettler Toledo), density (Mettler Toledo densimeter, precision 10^{-4}), and total soluble solids (TSS) measured as °Brix using an Atago RX-5000 digital refractometer at 20 °C.

Chemical Characterization of Citrus Juice

Titratable Acidity (TA)

Titratable Acidity (TA) was determined via titration with 0.1 N NaOH and phenolphthalein indicator, with results expressed as % citric acid (w/w).

Maturity index

Maturity Index was calculated as the TSS/TA ratio.

Ascorbic acid

Ascorbic acid content was determined by iodometric titration. A 10 mL sample was mixed with 10 mL of 1% KI and 10 mL of 1% H_2SO_4, then titrated with 0.01 N iodine solution in the presence of a starch indicator. Results were expressed as grams of ascorbic acid per liter of sample.

Dry Matter and Moisture

Dry Matter and Moisture were measured gravimetrically by drying samples at 105 °C for 24 h in a ventilated oven.

Ash Content

Ash Content was evaluated by calcination in a muffle furnace at 500 °C for 6 h.

Quantitative Analysis of Na+ and K+ Ions

Na+ and K+ Ions were determined using a Jenway PFP7 flame photometer, following calibration with standard salt solutions and appropriate sample dilution.

Quantification of Soluble Sugars Using High-Performance Liquid Chromatography

Total Soluble Sugars (glucose, fructose, and sucrose) were analyzed using HPLC (Supelcogel C-610H column, 30 °C, 0.1% H_3PO_4, flow rate 0.5 mL/min) and compared against calibration curves prepared from known sugar standards.

Isolation and Screening of Microorganisms

Pre-enrichment and Isolation of Microorganisms

Yeast isolates were obtained from traditional Moroccan sourdough, while acetic acid bacteria (AAB) were sourced from artisanal, unpasteurized apple vinegars. For yeast, samples were suspended in sterile physiological saline, subjected to serial dilution, and pre-enriched in YPD broth (glucose 20 g/L, yeast extract 5 g/L, peptone 10 g/L) supplemented with chloramphenicol (100 mg/L) to inhibit bacterial growth. For AAB, samples were enriched in GYEA medium (glucose 20 g/L, yeast extract 5 g/L, ethanol 3%, acetic acid 1%, plus essential salts). Enrichment cultures were incubated aerobically at 30 °C for 4 days at 130 rpm. Serial dilutions were then plated on solid YPD agar for yeast and GYEA for AAB. Plates were incubated at 30 °C (48–72 h for yeast; 3–5 days for AAB), and morphologically distinct colonies were sub-cultured to obtain pure isolates. All strains were maintained on their respective media and preserved in glycerol stocks at −20 °C.

Characterization and Performance Testing

The isolated microorganisms (yeast and AAB) were first characterized by observing their colony appearance (color, texture, elevation) and cell features under a microscope (shape, size, arrangement). To evaluate their performance, each isolate was grown in a liquid culture—yeast in YPD broth and AAB in GYEA—starting with a standardized amount of cells (optical density OD = 0.2). After incubating for 24–48 hours, the growth (biomass) was measured using a spectrophotometer (OD_{600} for yeast, OD_{540} for AAB), and the amount of ethanol or acetic acid produced was measured in the liquid (supernatant) using HPLC and titration methods.

Kinetic Study of Alcoholic and Acetous Fermentations

Alcoholic Fermentation

Batch fermentations were carried out in 250 mL Erlenmeyer flasks containing 50 mL of either YPD medium or citrus juice, inoculated with selected yeast isolates or Saccharomyces cerevisiae at a final concentration of 0.8 g/L. Fermentations were conducted under static conditions at 30 °C. Samples were taken at 4-hour intervals over a 24-hour period to monitor biomass production, residual glucose, ethanol, and glycerol concentrations. Ethanol levels were determined using both HPLC and an ebulliometer for validation. Fermentation was considered complete when CO_2 release visibly ceased.

Acetous Fermentation

Selected acetic acid bacteria (AAB) isolates were inoculated into filtered, fermented citrus musts containing approximately 5% (v/v) ethanol. Acetification was conducted under aerobic conditions in 250 mL Erlenmeyer flasks, each containing 150 mL of must and 50 mL of bacterial inoculum. Cultures were incubated at 30 °C with agitation at 130 rpm. Fermentation kinetics were monitored every 24 hours over a 6-day period by measuring optical density at 540 nm (OD_{540}), pH, and

titratable acidity. Acetic acid yield was calculated based on the conversion of ethanol in the substrate.

Pilot Plant-Scale Production and Start-Up Protocol

A pilot-scale semi-continuous acetous fermentation was conducted in a 500 L stainless steel acetifier (Pasto Gilson, France) with a working volume of 300 L. The bioreactor featured integrated aeration (20 L/min), temperature regulation (30–31 °C), and an internal recirculation system to preserve volatile aroma compounds.

Prior to scale-up, a 5 L preculture of AV22 selected AAB was prepared in a 6-L Infors lab-scale fermenter, cultivated under optimized conditions until OD540 = 0.4. A 60 L inoculum was then transferred into the acetifier containing ethanol- and acid-adjusted must (ethanol 5%, acetic acid 1%).

The fermentation strategy involved gradual feeding to maintain ethanol concentration and prevent inhibition. Titrable acidity, ethanol, OD540, pH, and bacterial load were monitored throughout the 13-day process. Semi-continuous operation was guided by adapted protocols (Mounir et al., 2016) to simulate industrial conditions.

Sensory Evaluation of Citrus Vinegar

A hedonic sensory analysis was conducted with 52 regular vinegar consumers (aged 18–65). Participants evaluated odor and taste intensity using a 9-point scale (1 = absence; 9 = highly pronounced). Each assessor was given 10 mL of Sanguinelli vinegar in blind-coded cups. Responses were subjected to Principal Component Analysis (PCA) and Cluster Variable Analysis using Minitab 18 to explore perception patterns (Cejudo-Bastante et al., 2016; Giuffrè et al., 2019).

Statistical Analysis

All experimental data were subjected to rigorous statistical evaluation to assess the significance of differences observed between microbial isolates, substrates, and fermentation yields. A one-way analysis of variance (ANOVA) was performed to determine significant differences among treatments.

In addition, a multi-criteria decision analysis (MCDA) approach was applied to rank the performance of the microbial isolates based on multiple simultaneous parameters, including biomass production, ethanol or acetic acid yields, and substrate tolerance.

All statistical analyses were conducted using a dedicated software package (Minitab 18®, Minitab Inc., USA), with the level of significance set at $p < 0.05$. Results are reported as mean values ± standard deviation from three independent replicates (n = 3). When appropriate, multiple comparisons were performed using Tukey’s post-hoc test.

RESULTS AND DISCUSSION

Characterization of citrus fruits

The four citrus varieties—Maroc Late, Sanguinilli, Pomelo, and Nadorcott—were analyzed following juice extraction to assess their morphological and physicochemical characteristics. These evaluations allowed for a comparative analysis among the varieties and with data reported in the literature. The characterization aimed to assess fruit quality and to determine the potential suitability of each variety for various applications, including bioethanol and acetic acid production, which will be discussed in subsequent sections. Additionally, the results offer insights into their

appropriateness for juice extraction and fresh consumption. A summary of the morphological traits observed is presented in Table 1.

Physical analysis and performance evaluation

The physical characteristics and performance metrics of the four citrus cultivars examined in this study are presented in Figure 1. These parameters were used to differentiate the cultivars and to evaluate their quality during the current growing season in selected regions of Morocco. A high juice yield is advantageous in the fruit processing industry, as it contributes to reducing production costs associated with citrus juice (Chen et al., 2023). Juice yield is influenced by both the cultivar and the extraction method employed. In the present study, a single juice extraction method was applied to three of the cultivars, while two different methods were necessary for Nadorcott due to its unique fruit structure. Additionally, extraction efficiency and juice loss during processing were assessed. The juice yield observed among the cultivars was relatively high overall. Maroc Late exhibited the highest yield at 56%, followed by Pomelo (53%), Sanguinelli (52%), and Nadorcott (46%). The comparatively lower yield from Nadorcott may be attributed to its smaller fruit size and the difficulty of juice extraction with the methods used. Its internal structure, characterized by segments that readily detach from the peel, may have also contributed to a less efficient extraction process.

Chemical properties and quality parameters of citrus juice from 4 cultivars

Titratable acidity

Citric acid is the predominant organic acid in citrus fruits, comprising approximately 70–90% of the total organic acid content (Lado et al., 2018). Its concentration can vary widely among cultivars, ranging from 4.43 to 140.5 g/L depending on variety and stage of ripeness (Coelho et al., 2021). Notably, acid content tends to decrease as fruits mature (Mbogo Gloria et al., 2010), making titratable acidity a useful indicator of harvest timing and fruit development.

In the present study, the cultivars analyzed fell within the lower end of the citric acid concentration spectrum, ranging from 4.43 to 18.9 g/L, as reported by Coelho et al. (2021). As shown in Figure 2, titratable acidity (TA), expressed as a percentage of citric acid, varied considerably among the four citrus cultivars. Pomelo had the highest TA at 0.81%, followed by Maroc Late at 0.75%. Sanguinelli and Nadorcott showed lower levels, at 0.35% and 0.29%, respectively. The lower acidity in Nadorcott and Sanguinelli may indicate more advanced ripening stages, as organic acid concentration typically decreases with maturity (Mbogo Gloria et al., 2010). These differences may influence the sensory perception of sourness and are relevant for processing and consumer preference.

Vitamin C or ascorbic acid

Ascorbic acid concentrations varied notably among the four citrus cultivars analyzed. Maroc Late had the highest content at approximately 0.56 g/L, followed by Sanguinelli (0.43 g/L), Pomelo (0.39 g/L), and Nadorcott (0.18 g/L). The differences were substantial, with Maroc Late containing more than three times the amount found in Nadorcott, and Sanguinelli containing more than twice as much. These results are shown in Figure 3. Although citrus fruits are generally rich in ascorbic acid, these findings emphasize that vitamin C content can be highly cultivar-dependent. And such differences can impact the nutritional labeling and marketing of citrus juices.

Determination of density, sodium, potassium, dry matter, moisture and ash contents

Figure 4 presents selected chemical properties of the citrus cultivars analyzed. Two mineral elements, potassium and sodium, were quantified. Maroc Late exhibited the highest potassium concentration, followed by Sanguinelli, Nadorcott, and Pomelo. In contrast, Nadorcott had the

highest sodium content at 290 mg/L, with Pomelo, Maroc Late, and Sanguinelli following in descending order. While sodium levels varied less markedly among the cultivars, potassium concentrations showed more pronounced differences. In all samples, potassium content was substantially higher than sodium. Moisture content across all cultivars exceeded 89.0%. Sanguinelli had the highest moisture level at 91.4%, followed by Maroc Late (90.32%), Nadorcott (89.9%), and Pomelo (89.2%). These values confirm the high water content typically found in citrus fruits. High moisture content is characteristic of citrus fruits and is associated with lower caloric density, an important nutritional factor. Ash content ranged from 2.32 g/L to 3.12 g/L among the four cultivars.

Determination of total soluble solids (TSS)As shown in the figure 5, total soluble solids (TSS) content varied among the citrus cultivars analyzed. Pomelo exhibited the highest TSS at 12.8%, while Sanguinelli had the lowest at 10.3%. These differences reflect variations in sugar accumulation, which is influenced by cultivar genetics, ripening stage, and environmental factors such as soil, water availability, and climate (Saini et al., 2022). Higher TSS levels typically enhance perceived sweetness and are a key indicator of fruit quality.

Maturity index and related quality parameters

The maturity index (TSS/TA ratio) ranged from 15.6 in Maroc Late to 43.08 in Nadorcott. Sanguinelli and Pomelo had intermediate values of 29.5 and 15.8, respectively. All values exceeded those reported in prior literature, suggesting advanced maturity at harvest or favorable environmental conditions.

The maturity index is widely used to evaluate the balance between sweetness and acidity, a key factor in flavor and consumer preference (Lado et al., 2018; Farag et al., 2020). Higher maturity index values generally reflect sweeter, less acidic fruits. In this study, Nadorcott, which had the highest index, also showed low TA and high pH—consistent with more mature fruit. Conversely, Maroc Late exhibited lower TSS/TA despite relatively high TSS, due to elevated acidity.

This pattern aligns with findings from Barry et al. (2003), who noted that water-deficit stress in arid growing conditions can lead to increased sugar accumulation via osmotic adjustment, raising the maturity index. Handaji et al. (2013) also reported a decline in organic acid content with advancing fruit maturity, supporting the observed trend in Nadorcott and Sanguinelli.

Additionally, pH values were slightly higher in Sanguinelli and Nadorcott (4.57 and 4.53) than in Maroc Late and Pomelo (3.87 and 3.88), mirroring the inverse relationship between pH and TA. Cultivars with higher pH generally exhibited lower acidity, further validating the use of TSS/TA as a reliable maturity and flavor indicator.

Total soluble solids by High Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) analysis was used to quantify the concentrations of sucrose, glucose, and fructose—known to be the most abundant soluble sugars in citrus fruits (Emmanouilidou and Kyriacou, 2017). The sugar concentrations varied slightly among cultivars, with total values ranging from 11.0 to 38.0 g/L as presented in Table 2.

Sucrose was the dominant sugar in all four cultivars, with the highest concentration observed in Pomelo, followed by Nadorcott, Sanguinelli, and finally Maroc Late, which recorded 19.4 g/L. Glucose was the second most abundant sugar, ranging from 11.0 g/L in Maroc Late to 24.9 g/L in Sanguinelli. Notably, Sanguinelli, Pomelo, and Nadorcott all exhibited glucose concentrations exceeding 20 g/L. Fructose was present in the lowest amounts across cultivars, with the highest value observed in Sanguinelli (21.0 g/L), followed by Pomelo (19.1 g/L), Nadorcott (17.8 g/L), and Maroc Late (12.2 g/L).

The glucose-to-fructose ratio was also assessed, and all cultivars met the generally accepted

threshold of >0.85 (Legua et al., 2022), with values ranging from 0.9 (Maroc Late) to 1.18 (Sanguinelli). Sanguinelli, Pomelo, and Nadorcott had ratios above 1, indicating a slight predominance of glucose over fructose. Overall, the distribution of sugars approximated a 1:1:2 ratio for glucose, fructose, and sucrose, respectively, aligning with trends reported in previous studies (Mbogo Gloria et al., 2010). Among the total sugar content, sucrose accounted for 41–50%, while glucose and fructose contributed 23–32% each, with only minor variation between cultivars.

Interestingly, while Coelho (2021) reported that cultivars with the highest levels of vitamin C, organic acids, and sugars also exhibited the highest total soluble solids (TSS), this trend was not confirmed in the present study. No clear correlation was observed between these parameters, suggesting cultivar-specific metabolic dynamics. Nonetheless, the combined accumulation of sugars and organic acids remains a central feature of citrus fruit maturation and is likely influenced by complex environmental and physiological factors.

Screening of yeast and acetic acid bacteria

The primary objective of this study was to isolate microbial strains suitable for the fermentation of citrus sorting reject juice, with the aim of producing bioethanol and acetic acid. A total of five yeast isolates were obtained from Moroccan traditional sourdough, while nine isolates of acetic acid bacteria (AAB) were recovered from two samples of traditional apple vinegar collected from different regions of Morocco.

Yeast isolates were cultivated using yeast extract peptone dextrose (YPD) medium, whereas glucose yeast extract calcium carbonate agar (GYEA) was used for the selective isolation of AAB. The yeast isolates were designated with the codes L1, L2, L3, L4, and LP. Acetic acid bacteria were labeled based on their vinegar source: those isolated from vinegar sample V1 were coded AV11, AV12, AV13, and AV14; those from sample V2 were designated AV21, AV22, AV23, AV24, and AV25.

Performance tests

Performance tests for yeast isolates

To identify the most efficient yeast strain for alcohol fermentation in vinegar production, a three-stage performance screening was conducted. All results from these stages are summarized in Figure 6.

Stage 1: Fermentation Profile on Standard Medium

In the initial screening, six yeast isolates—L1, L2, L3, L4, Lev2, Inc Lev1—and one commercial dry yeast (LS) were evaluated for ethanol, glycerol, and acetic acid production, as well as residual glucose and cell growth (OD600). Among all isolates, L3 produced the highest ethanol concentration (6.80 g/L), followed by L1 (6.14 g/L), Lev2 (5.97 g/L), L4 (5.45 g/L), L2 (5.44 g/L), Inc Lev1 (5.33 g/L), and LS (lowest at 5.33 g/L).

Glycerol was the second most abundant metabolite, ranging from 0.42 g/L (L3) to 0.64 g/L (Lev2). Acetic acid was produced in low amounts overall, with the highest level recorded in L2 (0.31 g/L), followed by Lev2 (0.25 g/L), L1 (0.24 g/L), L4 (0.21 g/L), Inc Lev1 (0.19 g/L), and both L3 and LS (0.17 g/L).

Residual glucose levels were lowest in Lev2 (0.00 g/L) and LS (0.32 g/L), indicating more complete sugar utilization, while L2 and L4 had the highest remaining glucose at 1.24 g/L and 1.10 g/L, respectively. Optical density (OD600) values, reflecting cell growth, ranged from 1.47 (LS) to 1.68 Abs (Inc Lev1).

Stage 2: Secondary Screening of Selected Isolates

Based on origin and initial performance, four isolates—L1, L2, L3, and L4—were selected for further testing. A shift in performance was observed, with L1 now producing the highest ethanol concentration (6.81 g/L), followed by L4 (5.98 g/L), L3 (5.36 g/L), and L2 (5.06 g/L). Other metabolites such as glycerol and acetic acid were not detected, possibly due to changes in metabolic conditions. As L2 had the lowest ethanol production in this stage, it was excluded from further testing. OD600 values ranged broadly, from 1.98 (LS) to 4.65 Abs.

Stage 3: Fermentation on Citrus Substrate

In the final stage, the remaining three isolates (L1, L3, and L4) and the commercial dry yeast (LS) were tested on Maroc Late citrus juice with an initial Brix of 11.5%. After 24 hours of fermentation, Brix levels decreased to 4.41% (L3), 4.51% (L1), 5.17% (LS), and 6.16% (L4), indicating variable sugar consumption. Interestingly, despite L3 showing the most significant drop in Brix, it yielded the lowest ethanol concentration (3.80% vol.). L1 and LS produced equal amounts of ethanol (3.95% vol.), while L4 achieved the highest ethanol concentration at 4.60% vol.

Yield Efficiency

Yield analysis revealed variation across the stages. In stage 1, yields ranged from 27% (LS) to 34% (L3). In stage 2, LS again showed the lowest yield (22%), while L1 reached 34%. In the final stage using citrus juice, the highest yield was recorded by L4 and L1 (40%), followed by LS (34%) and L3 (33%).

These results suggest that L4 and L1 are the most promising candidates for alcohol production from citrus sorting reject juice, offering high yields and ethanol concentrations under both synthetic and natural fermentation conditions.

Performance test for acetic acid bacteria

This test aimed to evaluate the ability of acetic acid bacteria (AAB) isolates to produce acetic acid and gluconic acid via the oxidation of ethanol and glucose, respectively. Simultaneously, the isolates' capacity to grow and tolerate acidic conditions was assessed in order to identify a suitable candidate for vinegar production in a bioreactor. The performance of the isolates was monitored over 48 hours of fermentation, and growth was evaluated through optical density measurements at 540 nm (OD540).

The isolates displayed distinct growth behaviors. AV12 demonstrated the highest biomass production throughout the fermentation period. AV11, AV22, and AV23 showed moderate growth, although AV22 presented a particular profile where biomass accumulation ceased around 40 hours, with no further increase observed. In contrast, AV13, AV21, AV24, and AV25 exhibited low biomass production for the duration of the assay. Despite its moderate growth, AV22 produced the highest concentration of acetic acid at 3.91% (w/v) after 48 hours, followed by AV21 at 2.15% (w/v). The remaining isolates produced lower concentrations in the following order: AV12, AV25, AV11, AV23, AV14, AV13, and AV24. Notably, AV22 achieved the highest acid production despite limited biomass formation, suggesting high oxidative efficiency relative to cell growth (Figure 7).

Under the experimental conditions used in this study, no gluconic acid production was detected in any of the isolates. This observation will be further investigated in subsequent phases of the work. Overall, the results indicate that AV22 is the most promising candidate for acetic acid production under acidic conditions, combining efficient acid yield with metabolic resilience.

Determination of the most performing isolates

To evaluate the performance of the isolated yeast and acetic acid bacteria (AAB), a one-way analysis of variance (ANOVA) was conducted. As shown in Table 3 for yeast and Table 4 for AAB,

the p-values obtained from the ANOVA tests were greater than 0.05 in all cases, indicating that there were no statistically significant differences among the isolates. Similarly, the F-values were low, further supporting the conclusion that the observed differences between group means were not statistically significant. Therefore, we do not have sufficient evidence to assert that the means of the isolates differ significantly in terms of their metabolic outputs.

Due to the lack of statistically significant differences, it was not possible to determine the best-performing isolates based solely on ANOVA results. Consequently, the selection was based on the average metabolite production observed across all experimental trials, including additional tests not detailed in this study. For yeast, the isolate with the highest average ethanol production was selected. For AAB, the isolate with the highest average acetic acid production, as well as the one that reached the highest peak value, was chosen. Based on these criteria, isolate L4 and isolate AV22 were identified as the most promising candidates for vinegar production.

Study of alcoholic fermentation kinetics of isolated strains and reference strain for ethanol production in synthetic medium

The dynamics of glucose consumption and metabolite production (ethanol and glycerol) during fermentation by yeast isolates and the reference strain Saccharomyces cerevisiae were monitored over a 28-hour period, with measurements taken every 4 hours (Figure 8).

A clear inverse relationship was observed between glucose concentration and the production of ethanol and glycerol. Glucose levels declined steadily, with complete consumption occurring at 16 hours for isolates L1 through L4, and at 28 hours for the reference strain LS. This indicates that the isolated strains metabolized glucose more rapidly than S. cerevisiae.

Ethanol production increased as glucose was consumed, reaching peak concentrations of 4.00 g/L (L1), 4.02 g/L (L2), 4.27 g/L (L3), 4.11 g/L (L4), and 4.13 g/L (LS). Among all strains, isolate L3 exhibited the highest ethanol yield, followed by LS, L4, L2, and L1. The observed decline in ethanol concentration following the depletion of glucose suggests that ethanol may have been utilized as a secondary carbon source by the yeast.

Glycerol production, known to be associated with osmotic stress regulation, peaked early in the fermentation process, particularly around the 4-hour mark. The highest initial glycerol concentrations were 0.66 g/L for L1 and 0.48 g/L for L4. Glycerol levels then declined and fluctuated alongside ethanol concentrations, stabilizing at final values of 0.16 g/L (L1), 0.14 g/L (L2), 0.05 g/L (L3), 0.06 g/L (L4), and 0.25 g/L (LS).In addition to the metabolic parameters discussed above, optical density (OD) was also measured as an indicator of biomass production. During the first 4 hours, all strains exhibited a lag phase, likely corresponding to the adaptation period and the onset of rapid cell multiplication. Notably, S. cerevisiae (LS) produced lower biomass compared to the sourdough isolates. Overall, the yeast isolates demonstrated a higher capacity for biomass accumulation, suggesting enhanced growth performance under the fermentation conditions tested.

Study of alcoholic fermentation kinetics of isolated strains of yeast in synthetic medium

The alcoholic fermentation kinetics of four isolated yeast strains (designated L1, L2, L3, and L4) were evaluated in a synthetic medium and compared to those of a reference industrial Saccharomyces cerevisiae strain (LS). The parameters monitored during the fermentation process included ethanol concentration, °Brix (as an indicator of sugar consumption), and pH variation (Figure 9).

The LS strain exhibited a rapid fermentation profile, characterized by the absence of a pronounced lag phase and a faster decline in °Brix values compared to the isolated strains. This suggests an enhanced metabolic capacity for sugar utilization. In contrast, isolates L1, L2, and L3 demonstrated

similar fermentation behaviors, marked by slower sugar consumption kinetics, which strongly suggests they may be genetically identical or highly similar. Isolate L4 displayed a slightly accelerated fermentation rate compared to L1–L3 but still followed a comparable kinetic pattern.

Throughout the fermentation process, pH levels remained relatively stable across all strains, ranging from 3.69 to 3.84. These values fall within the survival range reported for S. cerevisiae, which is typically between pH 2.75 and 4.25, though the optimal fermentation performance is noted between pH 4.0 and 4.25 (Moneruzzaman Khandaker et al., 2020). The consistent pH profiles suggest that none of the yeast strains induced significant acidification or alkalinization of the medium.

At 8 hours into fermentation, LS had produced 3.00% (v/v) ethanol, while L4 reached 1.44% (v/v). By the 19th hour, ethanol concentrations for L1, L2, and L3 reached 5.80% (v/v), whereas both LS and L4 achieved 6.40% (v/v). Maximum ethanol production was observed at approximately 29 hours for all strains, with a final ethanol concentration of 6.70% (v/v), starting from an initial °Brix of 15.64%. This plateau in ethanol levels, accompanied by the stabilization of °Brix values, indicates that the yeast strains were unable to completely metabolize all the fermentable sugars present in the medium, possibly due to nutrient limitation or inhibitory effects of accumulated ethanol.

These results collectively demonstrate that the isolated strains possess comparable fermentative capacities to the industrial S. cerevisiae strain LS in terms of ethanol yield. Therefore, from a bioethanol production perspective, the isolates are potentially viable alternatives to the commercial strain.

Kinetics of Acetous Fermentation by Acetic Acid Bacteria (AAB) in Synthetic Medium

The kinetics of acetous fermentation by selected acetic acid bacteria (AAB) isolates were assessed in a synthetic medium containing 5% (v/v) ethanol and 1% (v/v) acetic acid. Growth kinetics revealed significant variability among the isolates. Isolate AV25 exhibited the lowest biomass accumulation, with OD_{600} values considerably below those of the other strains. AV21 displayed a continuous and smooth growth curve, with biomass levels still increasing at the end of the 144-hour fermentation period, followed by AV23 and AV24. The isolates derived from source V1 (AV11, AV12, AV13, and AV14) exhibited intermediate growth profiles, with curves situated between those of AV22 and AV24, and showed relatively uniform kinetic behavior.

In terms of acid production, the performance profile differed from the growth kinetics. AV14 produced the highest acetic acid concentration (2.96% w/v), followed by AV24 (2.24% w/v). AV22 also demonstrated substantial acidification capacity (1.93% w/v), outperforming AV13. Isolates AV11 and AV12 followed closely in acetic acid yield, suggesting that the V1 isolates were particularly effective under the test conditions. In contrast, AV23, AV21, and AV25 produced the least acetic acid, consistent with their lower biomass levels.

A comparative analysis of growth and acid production trends highlights isolate AV22 as particularly notable. Its OD_{600} and titratable acidity curves were closely aligned, showing a steady, continuous increase without distinct stationary phases, indicating a well-adapted metabolic profile under the provided conditions.

In conclusion, the isolates from V1, especially AV14, demonstrated superior performance in acetic acid production, while AV22 and AV24 from V2 also showed promising kinetic and metabolic profiles. These results suggest that certain AAB strains, particularly from the V1 source, are well-suited for efficient acetous fermentation in synthetic ethanol-based substrates.

Study of acetous fermentation and its kinetics by AAB and the phenomenon of overoxidation of acetic acid

This experiment aimed to determine whether the acetic acid bacteria (AAB) isolates were capable of overoxidizing acetic acid to carbon dioxide and water following the complete oxidation of ethanol. The test was carried out using citrus must obtained from previous yeast fermentations, which, due to its low ethanol concentration, was unsuitable for distillation but ideal for studying AAB behavior in a natural medium. An additional goal was to indirectly assess whether the isolates could produce gluconic acid, which would not be expected if overoxidation of acetic acid occurred.

After approximately 9 to 10 days of fermentation, ethanol was fully converted to acetic acid, and a subsequent decline in total titratable acidity (TTA) was observed in some isolates (Figure 10), indicating overoxidation. The performance of all isolates—including initial ethanol concentration, final TTA, net acetic acid produced, and yield—is summarized in Table 4.

Although the variation in initial ethanol concentrations limits direct comparison of acid production efficiency, the results effectively demonstrate the metabolic behavior of each isolate under citrus must conditions. Several isolates began to oxidize acetic acid after the ethanol was depleted, confirming their capacity for overoxidation.

This behavior suggests that the isolates likely belong to genera such as Acetobacter, Komagataeibacter, or Gluconacetobacter, which are capable of assimilating and fully oxidizing acetic acid via the tricarboxylic acid (TCA) cycle and glyoxylate shunt—an ability known as acetate “overoxidation” (Lynch et al., 2019). In contrast, Gluconobacter and some other AAB genera lack the necessary TCA cycle enzymes (Deppenmeier and Ehrenreich, 2008; Mamlouk and Gullo, 2013) and therefore cannot perform overoxidation. Additionally, Gluconobacter is known for producing gluconic acid under high-sugar conditions, which was not observed in this study. This absence further supports the conclusion that the isolates are unlikely to belong to the Gluconobacter genus.

Bioethanol extraction

Distillation was performed on citrus fermented musts to recover bioethanol, and the results are summarized in Table 5. Fermentations were conducted using citrus juice substrates of varying initial Brix values (11.35% to 17.46%) to investigate the potential relationship between sugar concentration and ethanol yield.

However, the influence of Brix on bioethanol yield could not be conclusively evaluated, as several key fermentation co-products—such as glycerol, acetic acid, succinic acid, higher alcohols, and other volatile compounds—were not quantified. These byproducts may significantly affect the overall ethanol yield and fermentation efficiency.

The ethanol yield from distillation ranged from 4.82% to 7.99%. The alcohol content of the recovered distillates varied between 65.4% and 85.1%, while the density ranged from 0.838 to 0.898 g/cm³—higher than that of pure ethanol (0.789 g/cm³). This difference is attributed to ethanol’s strong affinity for water, making complete separation difficult without further dehydration steps, such as molecular sieving or azeotropic distillation, to produce fuel-grade anhydrous ethanol.

Small scale Vinegar production

Alcoholic fermentation

Prior to vinegar production via alcoholic and acetous fermentation, it is critical to consider the initial sugar concentration, particularly Brix, as it directly influences ethanol yield. Glucose, the preferred monosaccharide for yeast metabolism, is a key driver of fermentation efficiency. A low Brix level typically results in insufficient ethanol concentrations for effective vinegar production.

In this study, a preliminary alcoholic fermentation was performed using the selected citrus cultivar

(Citrus sinensis cv. Sanguinelli) with an initial Brix of 10.32%. This initial fermentation resulted in an average ethanol yield of 4.03% (v/v) across three replicates, which is below the minimum 5% (v/v) threshold generally required for vinegar production. Based on these results, sucrose was added to increase the fermentable sugar content.

Approximately 879 g of sucrose was incorporated into the citrus must, raising the Brix from 10.32% to 26.66%. Following sucrose addition, alcoholic fermentation achieved ethanol concentrations of 11.70% and 10.75% (v/v), making the substrate suitable for subsequent acetous fermentation (Table 6).

The alcoholic fermentation was carried out in triplicate using 2.5 L static glass bottles, while the acetous fermentation phase was conducted in a 10 L bioreactor under controlled conditions.

Semi-Continuous Acetous Fermentation

Prior to inoculation, the citrus must was diluted to an ethanol concentration of 5.55% (v/v) by mixing 2.5 L of the original must containing 11.70% (v/v) ethanol with an equal volume (2.5 L) of sterilized distilled water. This step was necessary to reduce the ethanol concentration below the inhibitory threshold of 6% (v/v).

The diluted must (5.0 L) was subsequently inoculated with 500 mL of a pre-culture of AV22, representing 11.8% of the total working volume (5.5 L). Fermentation was conducted in a 10 L stirred bioreactor under semi-continuous conditions, following a modified protocol based on the method illustrated in Figure 11. The first stage of fermentation lasted 168 hours, during which 55.5 g/L of ethanol was oxidized. Agitation was maintained at 50 rpm during this stage. Temperature varied from a minimum of 21.32 °C at the start to a maximum of 33.12 °C, remaining close to 30 °C for most of the process. Dissolved oxygen (DO) levels remained above 39% during the first 43 hours but dropped sharply to 5.2% thereafter, falling as low as 2% between 139 and 168 hours. Upon ethanol depletion, a low acetic acid yield of 2.96% (w/v) was obtained.

To initiate the second stage, 745 mL of fresh must containing 10.20% (v/v) ethanol was added to the reactor. This addition was calculated to minimize dilution of the existing acetic acid. Agitation was increased to 80 rpm at this stage. Oxidation proceeded rapidly, and a maximum acetic acid concentration of 4.10% (w/v) was reached after 25 hours. The third stage started with the addition of 700 mL of must, and continued for approximately 28 hours, resulting in a final acetic acid concentration of 5.21% (w/v). Due to elevated foam formation and a total acidity exceeding 5% (w/v), 1680 mL of vinegar was withdrawn, and an equivalent volume of fresh must was introduced. This marked the conclusion of the first semi-continuous fermentation cycle.

The addition of fresh substrate led to dilution of both acetic acid and bacterial biomass. During the fourth stage (second cycle), the acetic acid concentration initially decreased from 4.58% to 4.24% (w/v) over the first 19 hours. However, by the 314th hour, acidity had increased to 5.41% (w/v), ultimately reaching a maximum of 5.56% (w/v) after an additional 4.5 hours. Residual ethanol at this point was measured at 0.04% (v/v). The second cycle lasted approximately 95.5 hours.

Overall, the semi-continuous acetous fermentation process utilizing AV22 yielded a total of 8.40 L of vinegar, demonstrating the strain’s capacity for efficient ethanol oxidation under controlled conditions and without a discernible stationary phase.

Pilot Plant-Scale Production

of vinegar From an initial input of 200 kg of Citrus reticulata cv. Nadorcott (clementines), a total of 80 L of vinegar was produced following a 13-day semi-continuous acetous fermentation process. The final product exhibited a titrable acidity of 7.0% (w/v) and a residual ethanol concentration of 0.4% (v/v), meeting commercial vinegar standards.

The fermentation was carried out in a 500 L stainless steel acetifier with a working volume of 300 L, and was inoculated with 60 L of an Acetobacter sp. AV22 preculture. The gradual feeding strategy effectively sustained microbial activity and avoided ethanol inhibition throughout the process. Process monitoring confirmed stable pH, consistent bacterial growth (OD540), and progressive ethanol oxidation leading to the targeted acidity level.

These results validate the scalability of the fermentation process and the robustness of the AV22 strain under near-industrial conditions, supporting its suitability for commercial vinegar production from citrus by-products.

The final vinegar product is shown in Figure 12.

Sensory analysis of Sanguinelli vinegar

A sensory evaluation was conducted to assess the organoleptic properties and overall acceptability of vinegar produced from Citrus sinensis cv. Sanguinelli. A total of 51 untrained panelists participated in the analysis, which utilized a descriptive approach involving 25 sensory attributes—9 related to taste and 17 to aroma. The intensity of each descriptor was rated on a 9-point scale, where 1 indicated absence, 3 weak, 5 moderate, 7 pronounced, and 9 highly pronounced. The average scores for each descriptor are summarized in Table 7.

Among the taste descriptors, sour was the most pronounced, followed by pungent, astringent, salty, and bitter, which are consistent with the expected flavor profile of vinegar. For aroma, vinegar, citrus, fruity, alcoholic, and floral notes were the most commonly perceived. Less frequently cited attributes—such as spicy, umami, caramel, and leather—received scores close to absence, likely due to the participants' limited sensory training.

To better understand relationships among descriptors, Hierarchical Agglomerative Cluster Analysis (HACA) was performed using Minitab®. As shown in Figure 12, the dendrogram revealed nine main clusters based on the correlation distance between variables. The amalgamation schedule demonstrated a significant increase in inter-cluster distance at step 17, justifying the selection of 9 clusters. These clusters group descriptors with similar sensory perception and help reduce data complexity for interpretation.

These findings align with those reported by Giuffrè et al. (2019), in which astringent, pungent, salty, bitter, spicy, and umami were frequently associated with vinegar. Descriptors such as citrus, floral, and fruity further contribute to distinguishing citrus-based vinegars from conventional products. Interestingly, the mushroom note was often associated by panelists with Moroccan traditional bread, likely due to the contribution of yeast isolate L4, originally derived from Moroccan sourdough, suggesting microbial origin of some aroma compounds.

To further explore variable relationships, Principal Component Analysis (PCA) was conducted. Following Kaiser's criterion (eigenvalues > 1), nine principal components were retained, explaining 75.4% of the total variance. The first two components accounted for 55.2% of the total variability. The PCA biplot (Figure 13) shows all descriptors loading positively on PC1. Core vinegar attributes including sour, salty, astringent, pungent, and mushroom were clustered near the origin, indicating their central role in perception. Aroma descriptors such as citrus, floral, and fruity were more positively associated with PC2, reinforcing their importance in aroma differentiation.

Five distinct groups were identifiable, based on proximity and correlation across both dimensions. Positive descriptors closely associated with vinegar acceptability and citrus character were highlighted in green, while less favorable or less commonly perceived attributes appeared in red. Descriptors near the origin—particularly sour and pungent—were deemed central to flavor definition. The presence of the mushroom note, possibly of microbial origin, may be variably interpreted as desirable or off-putting depending on individual preferences.

Overall, the sensory profile of Sanguinelli vinegar demonstrates a strong balance of traditional vinegar characteristics with distinct citrus-derived notes, supporting its potential as a differentiated product in the vinegar market.

CONCLUSION

This study highlights the feasibility of transforming citrus processing sorting rejects juice into bioethanol and vinegar using indigenous microbial strains. Yeast isolate L4 demonstrated strong fermentative capacity, producing over 11% (v/v) ethanol in sucrose-supplemented citrus juice, while acetic acid bacterium AV22 achieved acetic acid concentrations exceeding 5.5% (w/v), meeting international vinegar quality standards. The integration of alcoholic and acetous fermentation within a semi-continuous system using local strains presents a viable and sustainable approach to valorizing agro-industrial residues.

The process showcases not only microbial robustness in non-sterile, natural substrates but also potential scalability for rural and peri-urban applications in Morocco. The favorable sensory profile of the final product further supports its commercial promise. However, limitations such as the lack of molecular identification of isolates, absence of fermentation kinetics under controlled conditions, and the need for life cycle assessment (LCA) data remain important considerations for future research.

Overall, this work contributes to circular bioeconomy strategies by coupling traditional fermentation knowledge with low-tech, adaptive bioprocessing. Future efforts should focus on molecular characterization of strains, optimization at pilot scale, and exploration of co-product recovery. Collaborations with local cooperatives and policymakers will be essential for successful scaling, positioning this model as a replicable pathway for sustainable food waste valorization.

Acknowledgments

We would like to thank the Moroccan Ministry of Higher Education, Scientific Research and Innovation and the OCP Foundation who funded this work through the APRD research program.

REFERENCES

Ademosun A.O. (2022). Citrus peels odyssey: From the waste bin to the lab bench to the dining table. Applied Food Research, 2: 100083.

Ademosun A.O., Oboh G., Passamonti S., Tramer F., Ziberna L., Boligon A.A., Athayde M.L. (2016). Phenolic composition of orange peels and modulation of redox status and matrix metalloproteinase activities in primary (Caco-2) and metastatic (LoVo and LoVo/ADR) colon cancer cells. European Food Research and Technology, 242: 1949–1959.

Albrigo G.L., L.L. Stelinksi, L.W. Timmer (2019). Citrus, 2nd ed.

Barry G.H., Castle W.S., Davies F.S., Littell R.C. (2003). Variability in Juice Quality of `Valencia' Sweet Orange and Sample Size Estimation for Juice Quality Experiments. Journal of the American Society for Horticultural Science, 128: 803–808.

Berk Z. (2016). Citrus fruit processing. Academic Press.

Cejudo-Bastante C., Castro-Mejías R., Natera-Marín R., García-Barroso C., Durán-Guerrero E. (2016). Chemical and sensory characteristics of orange based vinegar. Journal of Food Science and Technology, 53: 3147–3156.

Chen J., Luo W., Cheng L., Wu J., Yu Y., Li L., Xu Y. (2023). Influence of cultivar and turbidity on physicochemical properties, functional characteristics and volatile flavor substances of pomelo juices. Foods, 12: 1028.

Choi I.S., Lee Y.G., Khanal S.K., Park B.J., Bae H.J. (2015). A low-energy, cost-effective approach to fruit and citrus peel waste processing for bioethanol production. Applied Energy, 140: 65–74.

Coelho E.M., da Silva Haas I.C., de Azevedo L.C., Bastos D.C., Fedrigo I.M.T., dos Santos Lima M., de Mello Castanho Amboni R.D. (2021). Multivariate chemometric analysis for the evaluation of 22 Citrus fruits growing in Brazil's semi-arid region. Journal of Food Composition and Analysis, 101: 103964.

Deppenmeier U., Ehrenreich A. (2008). Physiology of acetic acid bacteria in light of the genome sequence of Gluconobacter oxydans. Journal of Molecular Microbiology and Biotechnology, 16: 69–80.

Emmanouilidou M.G., Kyriacou M.C. (2017). Rootstock-modulated yield performance, fruit maturation and phytochemical quality of 'Lane Late' and 'Delta' sweet orange. Scientia Horticulturae, 225: 112–121.

FAO (2021). Citrus Fruit Statistical Compendium 2020.

Farag M.A., Abib B., Ayad L., Khattab A.R. (2020). Sweet and bitter oranges: An updated comparative review of their bioactives, nutrition, food quality, therapeutic merits and biowaste valorization practices. Food Chemistry, 331: 127306.

Giuffrè A.M., Zappia C., Capocasale M., Poiana M., Sidari R., Di Donna L., Bartella L., Sindona G., Corradini G., Giudici P., Caridi A. (2019). Vinegar production to valorise Citrus bergamia by-products. European Food Research and Technology, 245: 667–675.

Gomes R.J., Borges M. de F., Rosa M. de F., Castro-Gómez R.J.H., Spinosa W.A. (2018). Acetic acid bacteria in the food industry: Systematics, characteristics and applications. Food Technology and Biotechnology, 56: 139–151.

Grigoraş C.-G. (2012). Valorisation des fruits et des sous-produits de l'industrie de transformation des fruits par extraction des composés bioactifs. Doctoral dissertation, Université d'Orléans; Universitatea Vasile Alecsandri din Bacău (România).

Handaji N., Benyahia H., Arsalane N., Ben Azouz A, Gaboun F. (2013). Evaluation pomologique et organoleptique de 34 variants d'oranges (Citrus sinensis (L.) Osbeck) issus de semis apomictique en essai dans la region du Gharb. Al Awamia, 127: 47-70.

Hasan S., Sardar R.I. (2021). A production of bioethanol through the bioconversion of water hyacinth: A review. Int. J. Adv. Chem. Res., 3: 25-33.

John I., Muthukumar K., Arunagiri A. (2017). A review on the potential of citrus waste for D-Limonene, pectin, and bioethanol production. International Journal of Green Energy, 14: 599–612.

Klein-Marcuschamer D., Oleskowicz-Popiel P., Simmons B. A., Blanch H.W. (2012). The challenge of enzyme cost in the production of lignocellulosic biofuels. Biotechnology and Bioengineering, 109: 1083–1087.

Lado J., Gambetta G., Zacarias L. (2018). Special Issue: Quality and safety of fruits and vegetables at harvest Key determinants of citrus fruit quality: metabolites and main changes 4 during maturation. Scientia Horticulturae, 239: 78-79.

Legua P., Modica G., Porras I., Conesa A., Continella,A. (2022). Bioactive compounds, antioxidant activity and fruit quality evaluation of eleven blood orange cultivars. Journal of the Science of Food and Agriculture, 102: 2960–2971.

Lynch K.M., Zannini E., Wilkinson S., Daenen L., Arendt E.K. (2019). Physiology of acetic acid bacteria and their role in vinegar and fermented beverages. Comprehensive Reviews in Food Science and Food Safety, 18: 587–625.

Mahmoud K.F., Ibrahim M.A., Mervat E.D., Shaaban H.A., Kamil M.M., Hegazy N.A. (2016). Nano-encapsulation efficiency of lemon and orange peels extracts on cake shelf life. American Journal of Food Technology, 11: 63–75.

Mamlouk D., Gullo M. (2013). Acetic Acid Bacteria: Physiology and Carbon Sources Oxidation. Indian Journal of Microbiology, 53: 377–384.

MAPMDREF (2020). Filière agrumicole, Ministère de l'agriculture. https://www.agriculture.gov.ma/fr/filiere/agrumicole

María Luzón-Quintana L., Castro R., Durán-Guerrero E. (2021). Biotechnological processes in fruit vinegar production. Foods, 10: 945.

Mbogo Gloria P., Mubofu Egid B., Othman Chande C. (2010). Post harvest changes in physico-chemical properties and levels of some inorganic elements in off vine ripened orange (Citrus sinensis) fruits cv (Navel and Valencia) of Tanzania. African Journal of Biotechnology, 9: 1809–1815.

Moneruzzaman Khandaker M., Aliyu Abdullahi U., Dogara Abdulrahman M., Afiza Badaluddin N., Suryati Mohd K. (2020). Bio-ethanol production from fruit and vegetable waste by using Saccharomyces cerevisiae. Bioethanol technologies, IntechOpen.

Mounir M., Shafiei R., Zarmehrkhorshid R., Hamouda A., Ismaili Alaoui M., Thonart P. (2016). Simultaneous production of acetic and gluconic acids by a thermotolerant Acetobacter strain during acetous fermentation in a bioreactor. Journal of Bioscience and Bioengineering, 121: 166–171.

Saini R.K., Ranjit A., Sharma K., Prasad P., Shang X., Gowda K.G.M., Keum Y.S. (2022). Bioactive compounds of citrus fruits: a review of composition and health benefits of carotenoids, flavonoids, limonoids, and terpenes. Antioxidants, 11: 239.

Stabnikova O., Wang J.Y., Bo Ding H., Joo-HwaTay (2005). Biotransformation of vegetable and fruit processing wastes into yeast biomass enriched with selenium. Bioresource Technology, 96: 747–751.

Suhag R., Kumar N., Petkoska A.T., Upadhyay A. (2020). Film formation and deposition methods of edible coating on food products: A review. Food Research International, 136: 109582.

Taherzadeh M.J., Karimi K.T. (2007). Acid-based hydrolysis processes for ethanol from lignocellulosic materials bioethanol review. BioResources, 2: 707-738.

Vasić K., Knez Ž., Leitgeb M. (2021). Bioethanol production by enzymatic hydrolysis from different lignocellulosic sources. Molecules, 26: 753.

Zema D.A., Calabrò P.S., Folino A., Tamburino V., Zappia G., Zimbone S.M. (2018). Valorisation of citrus processing waste: A review. Waste Management, 80: 252–273.

References