

**Gisou Raouf Mehrpour**

**Acetification: an approach to valorize non marketed fruits**



**UAAlg**

UNIVERSIDADE DO ALGARVE

Instituto Superior de Engenharia

2022

**Gisou Raouf Mehrpour**

**Acetification: an approach to valorize non marketed fruits**

**Master's in Food Technology**

**Supervisors**

Célia Maria Brito Quintas

Patrícia Alexandra Reis Nunes



**UAAlg**

UNIVERSIDADE DO ALGARVE

Instituto Superior de Engenharia

2022

## **Declaration of authorship of work**

I declare to be the author of this work, which is original and unpublished. Authors and works consulted are properly cited in the text and are included in the reference list.

---

(Gisou Raouf Mehrpour)

**Copyright©2022 Gisou Raouf Mehrpour**

The University of Algarve reserves the right for itself, in accordance with the provisions of the Portuguese Law Code. Copyright and Related Rights, to archive, reproduce and publish the work, regardless of the medium used, as well as to disseminate it through scientific repositories, as long as the respective author and publisher are given due credit.

This thesis is lovingly dedicated to my wonderful and supportive mom and dad, Giti and Javad, who have been my constant source of inspiration and have given me the drive and discipline to tackle a task with enthusiasm and determination. Without you I would have been unable to accomplish this phase of education. You have stood behind me all the way.

Thank you so much for everything!

I adore you, my dear parents!

## **ACKNOWLEDGEMENT**

Throughout the writing of this dissertation, I have received a great deal of support and assistance.

I would first like to thank my supervisors, Professor Célia Maria Brito Quintas and Professor Patrícia Alexandra Reis Nunes for their invaluable supervision, support and patience. The door to their office was always open whenever I ran into a trouble spot or had a question about my thesis. Your insightful feedback pushed me to sharpen my thinking and brought my work to a higher level.

I wish to show my appreciation to all my professors in the department of food Technology; Profs. Ana Cristina Oliveira Lopes Figueira, Gil Vicente da Conceição Fraqueza, Isabel Maria Carneiro Ratão, Jaime Miguel Costa Aníbal, Jorge Alberto dos Santos Guieiro Pereira, Ludovina Rodrigues Galego, Maria Dulce Carlos Antunes, Maria Margarida Cortês Vieira and Rui Mariano Sousa da Cruz. They all played an important role in my academic research and daily life.

I would like to express my gratitude to Professor Eduardo Bruno Oliveira Esteves, director of the department of Food Technology of ISE/ UAAlg, who has always been available to help, guide and support with patience and kindness throughout not only this work, but also the first day of my academic life in the University of Algarve.

I would like to thank David Santo, the food engineer and mentor of vinegar «Fateixa», where supplied uncountable bottles of vinegars used for this project freely and kindly.

I would also like to thank the Instituto Superior de Engenharia of the University of Algarve, which made this work possible by offering all the required conditions for its completion.

I wish to acknowledge the help provided by the laboratory technicians - Clarisse José Cardoso Ramalho, Neusa Maria da Silva Ferro Rodrigues and Vera Margarida de Deus Nunes Gonçalves.

Finally, I must express my very profound gratitude to my lovely parents and my one and only sister (Gilda), who have been of help morally and financially. Without their tremendous understanding, unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis, I would not be able to reach this accomplishment. Thank you, forever and ever.

## ABSTRACT

Four different vinegars (Fig enxario, Fig, Sweet potato and Grape) were collected from a vinegar production factory, Fateixa, located in Algarve, Portugal and were examined for different physico-chemical and antimicrobial characteristics.

Results of the physico-chemical experiments revealed that in the tested vinegar samples, the pH ranged from 2.83 to 3.49 and the titratable acidity was between 4.84% and 6.75%. Moreover, the lowest Brix value was found in Grape vinegar (4.80) while the highest value was in Fig vinegar (11.20). Total phenolic and total flavonoid contents ranged from 0.35 to 2.03 mg GAE/ml and 93.68-357.31 mg catechin/ml, respectively. Antioxidant activity revealed no significant difference between samples. Color properties showed that L\* values of the tested vinegars varied from 25.47 to 28.36, and values of a\* and b\* were found in the range of -0.49-0.48 and 2.01-3.23, respectively.

The antibacterial properties of the vinegars were evaluated against eight bacterial isolates, Gram-positive (*Staphylococcus aureus* ATCC 25923, *Listeria innocua* ATCC 33090, *Staphylococcus epidermidis* ATCC 12228 and *Bacillus cereus* ATCC 10876) and Gram-negative (*Cronobacter sakazakii* ATCC 29544, *Salmonella enterica* Typhimurium ATCC 14028, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) bacteria. The highest antibacterial activity was found in Grape vinegar, however in the case of *C. sakazakii* and *S. Typhimurium*, Sweet potato vinegar had the greatest growth-inhibitory effects. It was also observed that *P. aeruginosa* was the most sensitive strain, particularly against Grape vinegar.

Furthermore, antifungal activity of the vinegars against seven yeast strains (namely, *Candida albicans* ATCC 10231, *Candida albicans* ATCC 90028, *Cryptococcus neoformans* YPO 186, *Saccharomyces cerevisiae* PYCC 3507, *Pichia membranifaciens* PYCC 2489, *Zygosaccharomyces bailii* PYCC 4806 and *Debaryomyces hansenii* PYCC 2968) was examined. None of the vinegars showed fungicidal activity against *C. albicans*, *S. cerevisiae* and *Z. bailii*. On the contrary, growth of *D. hansenii* was considerably affected by all samples, while the highest level of growth-inhibitory effect was observed for Sweet potato vinegar. In conclusion, the bacteria tested were more sensitive to the vinegars than the yeasts.

**Keywords:** Vinegar, fruit wastes, antimicrobial activity, acetification, valorization, bioactive compounds

## **Resumo**

Os resíduos produzidos pelas indústrias agrícolas têm um efeito negativo no meio ambiente e no crescimento económico de qualquer país e a utilização desses resíduos resultará em bens naturais que poderão ser utilizados como matérias-primas de baixo custo. A aplicação adequada de resíduos de frutas pode ser implementada para melhorar a sustentabilidade ambiental, minimizando os impactos ambientais desses resíduos e para melhorar produtos alimentares com elevado valor nutricional. A produção de vinagre a partir de subprodutos do processamento de alimentos ou resíduos de frutas, é uma possibilidade atraente da gestão de resíduos com benefícios económicos.

Os vinagres são comumente produzidos a partir de vinho, uva, cerveja, cidra de maçã, entre outros. O processamento ocorre em duas fases que começa com a fermentação alcoólica dos hidratos de carbono e termina com a acetificação. É necessário caracterizar diferentes parâmetros dos vinagres produzidos para avaliação da sua qualidade e classificação. As qualidades sensoriais desempenham um papel importante na determinação da qualidade do vinagre, sendo a qualidade sensorial afetada principalmente pelo seu aroma e sabor. Na verdade, os compostos aromáticos voláteis e os polifenóis têm um impacto significativo nas características organolépticas do produto (cor, sabor e adstringência) junto com seus aspetos benéficos. Além disso, os vinagres têm vários benefícios para a saúde cientificamente comprovados. Em vez de prevenir doenças, vários componentes bioativos podem diminuir o risco de doenças. Os compostos polifenólicos, que além de sua ação antioxidante, também são responsáveis pela cor e adstringência do vinagre, têm despertado muita atenção nesse sentido. Apresentam propriedades antioxidantes, antibacterianas, anticancerígenas, previnem a obesidade, assim como doenças de hipertensão e contribui para regular os níveis do colesterol devido à presença de componentes bioativos, como ácido acético, ácido gálico, catequina, epicatequina, ácido clorogênico, ácido caféico, ácido p-cumárico e ácido ferúlico.

Os vinagres são também muito utilizados como conservantes de alimentos devido à sua capacidade de inibir o crescimento microbiano. O vinagre contém uma variedade de ácidos orgânicos,

incluindo ácido acético, que pode penetrar nas membranas celulares dos microrganismos e causar a morte celular.

Neste trabalho, foram analisadas quatro amostras diferentes de vinagre numa fábrica de vinagre, Fateixa, localizada no Algarve, Portugal (vinagre de Figo Enxario, vinagre de Figo, vinagre de Batata doce e vinagre de Uva). Primeiramente, este estudo visou avaliar as diferentes propriedades físico-químicas das amostras de vinagre mencionadas, incluindo pH, concentração de ácido acético (acidez total), sólidos solúveis totais (°Brix), análise de cor, compostos fenólicos totais e flavonóides totais e capacidade antioxidante.

Os resultados obtidos revelaram que o pH dos vinagres testados variou de 2,83 a 3,49. Além disso, a acidez total das amostras de vinagre foi determinada entre 4,84% e 6,75% de ácido acético, sem diferença significativa entre as amostras. Os valores Brix das amostras variaram de 4,80 a 11,20 e o menor valor de sólidos solúveis totais (°Brix) foi encontrado no vinagre de Uva (4,80), enquanto o maior valor foi observado no vinagre de Figo (11,2). No caso das propriedades bioativas das amostras de vinagre, os teores fenólicos totais variaram de 0.35 mg equivalente de ácido gálico (GAE)/ml a 2.03 mg GAE/ml, enquanto o maior valor foi encontrado no vinagre de Figo e o menor valor foi encontrado no vinagre de Batata doce. Por outro lado, os teores de flavonóides totais (TFC) das amostras de vinagre foram obtidos na faixa de 93,68-357,31 mg catequina/ml e entre as amostras de vinagre, o vinagre de Uva e o vinagre de Enxario de Figo apresentaram os maiores e os menores teores de TFC. As medições da atividade antioxidante não revelaram diferença significativa entre as amostras, a qual variou de 0,05 mg TE/ml a 0,39 mg TE/ml. As propriedades de cor mostraram que os valores L\* das amostras de vinagre testadas variam de 25,47 a 28,36, e os valores de a\* e b\* foram encontrados na faixa de -0,49-0,48 e 2,01-3,23, respetivamente, tendo o vinagre de Batata doce obtido o maior valor L\*.

O presente estudo também se concentrou na avaliação da qualidade microbiana das amostras de vinagre através do estudo dos seguintes parâmetros: microrganismos aeróbios mesofílicos, bactérias ácido-lácticas, fungos filamentosos, leveduras fermentativas e bactérias acéticas. Os resultados obtidos permitiram concluir que todos os parâmetros microbiológicos se encontravam abaixo dos limites de deteção.

Outra parte do presente trabalho visou avaliar a atividade antimicrobiana de vinagres por meio do método de Kirby-Bauer, também conhecido como difusão em disco de agar. Para este propósito,

as propriedades antimicrobianas dos vinagres foram avaliadas contra 15 microrganismos (*Staphylococcus aureus* ATCC 25923, *Listeria innocua* ATCC 33090, *Cronobacter sakazakii* ATCC 29544, *Salmonella enterica* Typhimurium ATCC 14028, *Escherichia coli* ATCC 25922, *Staphylococcus epidermidis* ATCC 12228, *Bacillus cereus* ATCC 10876, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231, *Candida albicans* ATCC 90028, *Cryptococcus neoformans* YPO 186, *Saccharomyces cerevisiae* PYCC 3507, *Pichia membranifaciens* PYCC 2489, *Zygosaccharomyces bailii* PYCC 4806 and *Debaryomyces hansenii* PYCC 2968). Geralmente, a maior atividade antibacteriana foi encontrada em amostras de vinagre de Uva e no caso de *C. sakazakii* ATCC 29544 e *S. Typhimurium* ATCC 14028, o vinagre de Batata doce teve os maiores efeitos inibidores de crescimento. Observou-se também que *P. aeruginosa* ATCC 27853 foi a espécie mais sensível, principalmente contra o vinagre de uva.

Na última parte deste trabalho avaliou-se a atividade antifúngica das amostras de vinagre contra algumas leveduras, incluindo *C. albicans* ATCC 10231, *C. albicans* ATCC 90028, *C. neoformans* YPO 186, *S. cerevisiae* PYCC 3507, *P. membranifaciens* PYCC 2489, *Z. bailii* PYCC 4806 e *D. hansenii* PYCC 2968. Nesta parte do trabalho, observou-se que nenhuma amostra de vinagre apresentou atividade fungicida contra *C. albicans* ATCC 10231, *S. cerevisiae* PYCC 3507 e *Z. bailii* PYCC 4806. Pelo contrário, o crescimento de *D. hansenii* PYCC 2968 foi consideravelmente afetado por todas as amostras de vinagre, enquanto o nível mais alto de efeito inibidor de crescimento foi observado para o vinagre de Batata-doce (14,33 mm). De um modo geral, concluiu-se que as bactérias testadas são mais sensíveis aos vinagres em estudo do que as leveduras.

**Palavras-chave:** Vinagre, resíduos de fruta, atividade antimicrobiana, acetificação, valorização, compostos bioativos

## List of Figures

<b>Figure 1.1.</b> The formation of acetic acid from ethanol by AAB (Vidra and Németh, 2017).....	27
<b>Figure 1.2.</b> Slow ("Orléans") vinegar making in a barrel (adapted from Bekatorou, 2020).....	34
<b>Figure 1.3.</b> Types of quick vinegar making vats with wood shavings (adapted from Bekatorou, 2020) .....	35
<b>Figure 1.4.</b> A modern submerged fermentation acetator (based on the Frings technology) (adapted from Bekatorou, 2020).....	38
<b>Figure 2.1.</b> Commercialized vinegar samples used in this study.....	45
<b>Figure 2.2.</b> pH-Meter (Crison, Model Basic 20, Barcelona, Spain).....	46
<b>Figure 2.3.</b> Endpoint color in acetic acid measurement using titration method.....	47
<b>Figure 2.4.</b> Abbe refractometer (Atago, 1T, Japan).....	48
<b>Figure 2.5.</b> Spectrophotometer (Spectronic GENESYS 10).....	50
<b>Figure 2.6.</b> Cooling down the autoclaved media in water bath with temperature adjustment at 50°C.....	53
<b>Figure 3.1.</b> Plate Agar samples incubation: a) AAB, filamentous fungi and yeasts samples at 25°C and b) Mesophilic Aerobic Microorganisms and LAB samples at 30 °C.....	62
<b>Figure 3.2.</b> Antibacterial activity of Grape vinegar and Fig vinegar against <i>S. Aureus</i> ATCC 25923.....	64
<b>Figure 3.3.</b> Antibacterial activity of A) Grape vinegar and B) Sweet potato vinegar against <i>S. epidermidis</i> ATCC 12228.....	64
<b>Figure 3.4.</b> Comparison between the growth inhibition of the vinegar samples studied in this work: A) Fig enxario vinegar, B) Fig vinegar, C) Sweet potato vinegar, D) Grape vinegar, and E) Erythromycin against <i>L. innocua</i> ATCC 33090.....	65
<b>Figure 3.5.</b> Antibacterial activity of vinegar samples studied in this work: A) Fig enxario vinegar, B) Fig vinegar, C) Sweet potato vinegar and D) Grape vinegar against <i>C. sakazakii</i> ATCC 29544.....	66

**Figure 3.6.** Comparison between the growth inhibition by A) vinegar samples and B) antibiotics against *P. aeruginosa* ATCC 27853.....68

**Figure 3.7.** Antifungal activity of vinegar samples A) Fig enxario vinegar, B) Fig vinegar, C) Sweet potato vinegar and D) Grape vinegar against *D. hansenii* PYCC 2968.....73

## List of Tables

**Table 1. 1.** Physiological properties and characteristics of acetic acid bacteria (Yamada, 2016).  
..... 29

**Table 3. 1** Physicochemical properties of vinegar samples from Algarve region.....57

**Table 3. 2.** Bioactive properties of vinegar samples from Algarve region. ....59

**Table 3. 3.** Color parameters of vinegar samples from Algarve region. ....60

**Table 3. 4.** Enumeration of microorganisms in the vinegars (Fig enxario, Fig, Sweet potato and Grape vinegar) (CFU/ml).....61

**Table 3. 5.** Antibacterial activity of the vinegar samples from Algarve region against some bacterial pathogens (inhibition zone mm).....70

**Table 3. 6.** Antibacterial activity of different antibiotics against some bacterial pathogens .....71

**Table 3. 7.** Antifungal properties of the vinegar samples from Algarve region against some yeasts  
.....75

**Table 3. 8.** Antifungal properties of antimycotics against some yeasts (inhibition zone mm) .....76

# Table of content

1.	STATE OF THE ART .....	15
1.1.	<b>Agricultural wastes and importance of their management and reutilization .....</b>	<b>16</b>
1.1.1.	Agricultural wastes .....	16
1.1.2.	Fig ( <i>Ficus carica L.</i> ) .....	16
1.1.3.	Sweet potato ( <i>Ipomoea batatas</i> ) .....	17
1.1.4.	Grape ( <i>Vitis vinifera L.</i> ) .....	20
1.1.5.	Fruit waste management.....	22
1.2.	<b>Vinegar .....</b>	<b>23</b>
1.2.1.	History and definition of vinegars .....	23
1.2.2.	Biochemistry and substrate.....	24
1.2.2.1.	Alcoholic fermentation .....	24
1.2.2.2.	Acetification .....	25
1.2.3.	The industrial process of vinegar production .....	31
1.2.3.1.	Open vat process (Orléans Method).....	32
1.2.3.2.	The Quick/ Generator Method .....	34
1.2.3.3.	The Modern Rapid Submerged Methods.....	36
1.2.4.	Vinegar quality.....	38
1.2.5.	Bioactivities and Health Benefits of Vinegar .....	40
1.3.	<b>Objectives .....</b>	<b>43</b>
2.	<b>MATERIALS AND METHODS .....</b>	<b>44</b>
2.1.	<b>Sample collection .....</b>	<b>45</b>
2.2.	<b>Physico-chemical characterization .....</b>	<b>45</b>
2.2.1.	pH measurement .....	45
2.2.2.	Acetic acid measurement .....	46
2.2.3.	Determination of total soluble solids (°Brix).....	47
2.2.4.	Color measurement .....	47
2.2.5.	Determination of total phenolic content (TPC) .....	48
2.2.6.	Determination of total flavonoid content (TFC) .....	49

<b>2.3. Microbiological characterization .....</b>	<b>50</b>
2.3.1. Microbial quality evaluation .....	50
2.3.1.1. Dilution preparation .....	51
2.3.1.2. Enumeration of mesophilic aerobic microorganisms .....	51
2.3.1.3. Enumeration of total viable acetic acid bacteria (AAB) .....	51
2.3.1.4. Enumeration of Lactic acid bacteria (LAB) .....	52
2.3.1.5. Enumeration of fungi .....	52
2.3.1.6. Enumeration of yeasts .....	53
2.3.2. Antimicrobial activity .....	54
2.3.2.1. Microbial sample collection .....	54
2.3.2.2. Antimicrobial activity assessment experiments .....	54
<b>2.4. Statistical analysis .....</b>	<b>55</b>
<b>3. RESULTS AND DISCUSSIONS .....</b>	<b>56</b>
<b>3.1. Physico-chemical analysis .....</b>	<b>57</b>
3.1.1. Physico-chemical properties.....	57
3.1.2. Bioactive properties .....	57
3.1.3. Color parameters.....	59
<b>3.2. Microbial analysis .....</b>	<b>60</b>
3.2.1. Microbial quality evaluation.....	60
3.2.2. Antibacterial activity of the vinegar samples .....	63
3.2.3. Antifungal activity of the vinegar samples .....	72
<b>4. CONCLUSION .....</b>	<b>77</b>
<b>5. FUTURE PERSPECTIVES .....</b>	<b>79</b>
<b>6. BIBLIOGRAPHIC REFERENCES .....</b>	<b>81</b>

## LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
Na <sub>2</sub> CO <sub>3</sub>	Sodium Carbonate
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
N	Normality
N <sub>2</sub>	Nitrogen
nm	Nanometer
mm	Milliliter
OH	Hydroxyl
MHA	Müller-Hinton Agar
SD	Standard Deviation
TA	Titrateable Acidity
TE	Trolox Equivalent
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
TAC	Total Antioxidant Capacity

CFU	Colony Forming Unit
EQ	Equivalent
G	Gram
I	Inhibition
μg	Microgram
μg/Disc	Microgram Per Disc
μg/ml	Microgram Per Milliliter
% V/V	Percent Volume By Volume
% W/W	Percent Weight By Weight
R <sup>2</sup>	Coefficient Of Determination
M	Molarity
AAB	Acetic acid bacteria
LAB	Lactic acid bacteria

# **1. State of the art**

## **1.1. Agricultural wastes and importance of their management and reutilization**

### **1.1.1. Agricultural wastes**

Humankind has been producing wastes in various forms throughout the history. Modernization, rapid population growth and resource constraints resulted in the more complex forms of wastes in the environment (Atinkut *et al.*, 2020; Christy *et al.* 2014). The occurrence of different pollutions in soils and aquatic resources are the most important drawbacks of technological advancements and modernization. Among the different environmental pollutants, food wastes affect both the environment and human societies (Eriksson & Spångberg, 2017).

According to Plazzotta *et al.* (2017), in underdeveloped countries, due to seasonality that results in unsaleable gluts and the lack of effective conservation measures for perishable products, wastes are mostly created in agricultural production, post-harvest, and marketing phases, however, developed countries are also challenging by waste produced in agricultural industries. These produced wastes are shown to be a great source of different compounds from the nature and their utilization will result in natural goods with low-cost starting materials (Manzocco *et al.*, 2016).

### **1.1.2. Fig (*Ficus carica* L.)**

Figs (*Ficus carica*) are among the oldest cultivated plants in the mankind history (Hussain *et al.*, 2021a; Veberic & Mikulic-Petkovsek, 2016). They are members of the *Moracea* family which has about 60 genera and about 2000 species (Zolfaghari *et al.*, 2019) and originally belong to east of Mediterranean region and west of Asia (Hmimsa *et al.*, 2017; Ighbareyeh *et al.* 2018; Liu *et al.* 2019). Based on the United Nations' Food and Agriculture Organization, global fig productivity remains steady, with an average around 1.1 million tons per year during the last decade. Egypt, Turkey, Iran, Algeria, and Morocco supplied over 70% of the world's fig market in 2013 (FAOSTAT, 2019).

The cultivation of fig fruits and their great economic worth have been linked to their recognized health-supporting substances as well as their outstanding flavor and nutrient benefits (Veberic & Mikulic-Petkovsek, 2016; Khadivi *et al.*, 2018). Most of the *F. carica* species are rich in valuable compounds like phenolics, organic acids and different volatile compounds as well as other compounds such as fructose, glucose, sucrose and anthocyanins (Hussain *et al.*, 2021a) and

according to Jeong and Lachance (2001), they may contain phytosterols. The major bioactive components in this plant are flavonoids, and several studies revealed that fig extracts have exhibited biological activities (Bouyahya *et al.*, 2016). Based on various works, different polysaccharides from this fruit have antioxidant, antispasmodic and antitumor properties (Solomon *et al.*, 2006; Gilani *et al.*, 2008; Lansky *et al.*, 2008; Yang *et al.*, 2009).

However, fully ripe fig has the highest nutritional value, while, in this stage the fruits are very delicate and particularly vulnerable to harm (Allegra *et al.*, 2018). Consumers prefer fresh figs at the tree ripe maturity stage (Hussain *et al.*, 2021a; Crisosto *et al.*, 2010), color of skin and genotype (Çalışkan & Aytekin Polat, 2011). The fresh fig fruits have very short shelf life, about 7-8 days if refrigerated (Lo Turco *et al.*, 2020). They are highly vulnerable to physio pathological disorders such as softening and skin cracking during the post-harvest period (Allegra *et al.*, 2018; Kong *et al.*, 2013).

### **1.1.3. Sweet potato (*Ipomoea batatas*)**

Sweet potato (*Ipomoea batatas* (L.) Lam.) is an herbaceous plant and one of the important tuber crops grown in the tropics that belongs to the family Convolvulaceae (Bhattarai *et al.* 2021; Lebot, 2020). Sweet potatoes are native to South America (Mohanraj R. 2018). It was brought to Europe by Columbus, afterwards it spread to other areas of the world (Milind and Monika, 2015). Every year, more than 105 million metric tons of sweet potatoes are harvested across the world, while Asian and African countries, particularly China, contributing the majority of them (FAOSTAT, 2020).

Sweet potato (*Ipomoea batatas*) is a crop with particular qualities, including ease of cultivation for both large and small farms, high resistance, minimal production costs, and adaptability to a wide range of climates, making it valuable for developing-country agriculture (Nogueira *et al.*, 2018). The sweet potato crop has a remarkable benefit in that it can be grown all year in tropical and subtropical environments where warm weather is abundant, giving it a leg up in the event that staple crops, like cereals, are restricted by drought (Mohanraj, 2017).

Sweet potato cultivars have been found in a variety of growing areas. It obviously differs in shape, size, weight, and color depending on type, cultivar, and climate, which can also create variances in carotenoids content (Wang *et al.*, 2018; Dak *et al.*, 2016).

Sweet potato skins come in a variety of colors, including white, cream, yellow, orange, pink, red, and purple (Alam, 2021). Flesh hues vary from white to cream, yellow, orange, and even purple (Alam *et al.*, 2016; 2020; Wang *et al.*, 2018). According to Zannou *et al.* (2017), this coloration is indicative of the presence of red anthocyanins and their respective glucosides, whilst the orange coloration is due to the presence of carotenoids.

Park *et al.* (2016) argues light-colored cultivars with greater starch content, such as white and yellow fleshed, are valuable for starch producers. Orange, yellow, and purple-fleshed ones, on the other hand, are valued for their high levels of  $\beta$ -carotene (a precursor to vitamin A), anthocyanin, phenolic acid, and flavonoid content. Furthermore, orange-fleshed sweet potato has been shown to increase vitamin A levels in diets, making it effective against micronutrient deficiencies (Wang *et al.*, 2018).

Sweet potatoes contain a wide variety of macro and micronutrient. They are good source of vitamins (such as vitamin A, vitamin C, vitamin B complex and vitamin E), minerals (particularly potassium, sodium and manganese) and phytochemical, particularly polyphenols (such as chlorogenic acid); the bio-constituents in sweet potato have antioxidant and anti-inflammatory properties (Zengin *et al.*, 2017).

Sweet potatoes include a large number of bioactive compounds that contribute to the health advantages provided by them (Mohanraj 2017). Wang *et al.* (2018) and Shekhar *et al.* (2015) reported that the bioactive compounds differ among white-fleshed and orange-fleshed sweet potatoes.

According to Mohanraj (2017), phenolic compounds, such as phenolic acids and anthocyanins, are quite predominant in purple-fleshed varieties, while in orange-fleshed varieties predominant compounds are  $\alpha$ -carotene,  $\beta$ -carotene, and  $\beta$ -5 cryptoxanthin. These bioactive compounds give sweet potatoes unique flesh colors such as cream, yellow, orange, and purple (Sochinwechi Nwosisi *et al.*, 2017).

Even though sweet potato has a significant role in the supply of food, its consumption is assumed to be dropping as income rises, a tendency typically linked to urbanization, because it is regarded as a "poor man's meal" in part, but particularly owing to a lack of post-harvest processing and storage facilities (International Potato Center, 2021).

On an average, losses of around 15–65% of sweet potato have been estimated during all phases of the supply chain (Abewoy, 2021) which are due to its high moisture content, fragility, and pest-attractiveness (P *et al.*, 2019).

A study by Bhattarai *et al.* (2021) revealed that the skin of sweet potato roots is thin, delicate which is high in moisture (60–70%) and free in sugar (4–15%). They also reported that having a high respiratory rate immediately upon harvest, leading the texture to soften as a result of the heat output, making sweet potato a perishable crop (Bhattarai *et al.*, 2021). The shelf life of sweet potato roots ranges from a few days to a few weeks, depending on the variety, harvest maturity, and storage circumstances) (P *et al.*, 2019). Sweet potato tubers cannot last long after harvest if not treated properly. According to Zannou *et al.* (2017), sweet potatoes are usually stored in a dry, cool and ventilated chamber and after 3-6 weeks, their quality degrades by becoming fibrous, rot and germinate. However, their study also revealed that tubers can be stored for up to 6 months in the certain conditions consisting of a preliminary treatment of a few days in a hot and humid environment (29 °C and 90% humidity) followed by storage in between 13 and 16 °C and 85-90 % relative humidity (Zannou *et al.*, 2017). Sweet potato storage is not practiced in many parts of the world, due to its limited shelf life (P *et al.*, 2019). According to Bhattarai *et al.* (2021), one of the most prevalent reasons of rotting after harvest is lack of a suitable, technically verified and tested storage method. Furthermore, biochemical and physiological reactions take place during the long-term storing of sweet potato tubers, resulting in qualitative and quantitative alterations (Abidin *et al.*, 2016).

Therefore, it is important to improve the utilization and consumption rate of sweet potatoes through innovative diversification products that are attractive, nutritious, and have added value with simple technologies. Producing vinegar using sweet potato in a high-efficiency liquid fermentation process is a good way to improve vinegar manufacturing to meet the current market demand.

#### 1.1.4. Grape (*Vitis vinifera L.*)

Grape (*Vitis vinifera L.*) is one of the world's most frequently cultivated crops, with over 75 million tons produced each year; around 41% of grapes are grown in Europe, 29% in Asia, and 21% in the Americas (Colombo *et al.*, 2019). According to Ghan *et al.* (2015), there are more than 5000 distinct cultivars of grapes in the globe. Approximately 45 % of grape crop is used as such or as fresh derivatives, with the remaining 55 % fermented for wine making (FAO & OIV, 2016).

Grape production is found on every arable continent around the world. Hussain *et al.* (2021b) revealed grapes can survive in a broad range of climates and are cultivated well in temperate and tropical climates at elevations ranging from 1200 to 3200 feet above sea level.

With yearly output of 3 million metric tonnes from an area of 1.3 thousand hectares, China is the biggest grape grower, followed by India. Viticulture has been one of the most important socio-economic activities in Portugal, with an average of 195 thousand hectares of planted land earmarked for wine production in 2016 (State of the Vitiviniculture World Market, 2017). Portugal is the world's 11th largest wine producer, with 6.600 million hectoliters produced in 2017 (Aurand, 2017) and the 9th largest exporter, with 727 million Euros in 2016 (State of the Vitiviniculture World Market, 2017).

Grape (*Vitis vinifera L.*) contains a number of nutritional and functional compounds. Grapes are high in antioxidant phytochemicals such as phenolics, flavonoids, anthocyanins, resveratrol, and carotenes, as well as other beneficial substances for human health (Bunea *et al.*, 2012; Flamini *et al.*, 2013; Lebot, 2020). Sagdic *et al.* (2011) reported that the total extractable phenolics, in fresh grapes, are mostly dispersed in the skin (28–35%) and seeds (60–70%), as opposed to the pulp (10%), and the phenolics in the skin are transmitted into the final product (Kocabey *et al.*, 2016).

Grape consumption has been linked to a variety of beneficial health impacts, namely cardioprotective, anti-inflammatory, anti-carcinogenic, antiviral, and antibacterial properties, which are due to their high bioactive chemical content (Colombo *et al.*, 2019; Georgiev *et al.*, 2014; Ras *et al.*, 2013; Rasines-Perea and Teissedre., 2017; Vaisman and Niv, 2015; Xia *et al.*, 2010).

On the other hand, it is undeniable that grapes are highly perishable commodity, therefore storage time is limited, and challenges arise during postharvest storage and distribution (Baraiya *et al.*,

2016). Weight loss, color alterations, accelerated softening, and rachis browning are examples of quality losses (Sousa *et al.*, 2013) that contribute to a reduction in shelf-life. According to Baraiya *et al.* (2016) table grapes, like many other fruits, go through variety of physicochemical, biochemical and microbiological changes during storage, which accelerates ripening process and reduces shelf life.

A study by Söylemezoğlu (2003) revealed that water loss and decay-causing fungus are the two most critical elements influencing the length and quality of table grape storage. This author also mentioned to necessity of a high relative humidity to reduce moisture loss and keep the stem in excellent condition, in addition to the requirement of a low temperature to limit the respiration rate and, as a result, to minimize water loss (Söylemezoğlu 2003).

According to Food and Agriculture Organization of the United Nations (FAO) reports, there is a significant lack of marketing infrastructure in developing countries, consequently postharvest losses of fresh items can reach up to 50% of total production (FAO, 2011). Losses of this scale have a significant economic impact on the food production chain, particularly primary farmers (Pina-Barrera *et al.*, 2019). Grapes, among other berry fruits, contain a variety of nutrients, including vitamins, minerals, carbs, edible fibers, and phytochemicals (Pina-Barrera *et al.*, 2019). Therefore, in addition to apply innovative postharvest technologies, it is important to improve the utilization rate of grapes through novel products with simple and high-efficiency technology. Fruit vinegars contain many compounds with antioxidant properties, which may originate from the source material (i.e., fruit). A study by Antoniewicz *et al.* (2021) showed that the natural fermentation is a vinegar-making technology that uses the microbiota found naturally in the plant material. The grape microbiome is diverse, including considerable range of yeasts, bacteria, and filamentous fungi representing a wide spectrum of organisms (Antoniewicz *et al.*, 2021), which play an important role in the fermentation process, metabolizing grape sugars and affecting the development of a variety of secondary metabolites that determine the fermented product's final quality (Roullier-Gall *et al.*, 2020). Correspondingly, producing vinegar using non-marketed grapes by fermentation process could be considered as an affordable mean of agricultural by-products valorization.

### **1.1.5. Fruit waste management**

Fruit and vegetable waste (FVW) productivity has grown dramatically in recent years due to the rapid expansion of intensive agricultural operations. Plazzotta *et al.* (2017) observed that the fruit and vegetable industry produce a huge amount of trash. FVW can be created in different stages throughout the food supply chain, encompassing both pre- and post-consumer phases (Panda *et al.*, 2016). Waste production of fruit and vegetable is highly more than all types of other foods and may reach up to 60%, according to estimations made by FAO (2011). In industrialized countries, due to the overproduction and non-compliance with retailer quality requirements, FVW is mostly created before reaching customers. Fruit and vegetables that are tiny in size or misshaped are usually referred to as substandard (Plazzotta *et al.* 2017). According to a study by Sagar *et al.* (2018), seeds, skins, rinds, and pomaces of the fruits that make up the majority of the wastes of these products, are rich in different bioactive components like as polyphenols, carotenoids, vitamins, fibers, oils and enzymes.

In view of the fact that FVW are highly biodegradable, they cause serious environmental impacts as well as a loss of valuable organic compounds and economical expense to businesses (Esparza *et al.*, 2020; Plazzotta *et al.* 2017). As a result, much attention has been paid in recent years to the creation of policies and techniques for its management (Esparza *et al.*, 2020). According to Papargyropoulou *et al.* (2016), FVW management refers to all actions associated to preventing, minimizing or recycling these wastes along with the manufacturing and consumption cycle. Fruit waste management not only minimizes the pollutions occurring from these sources, but also reduces the raw material taken from the Earth (Esparza *et al.*, 2020).

Fruit waste is not appropriate for burning due to its high moisture content, however, because of the presence of organic components, stacking or landfilling will generate a considerable amount of leachate, resulting in major environmental contaminations (Esparza *et al.* 2020; Bhatt *et al.*, 2017). As a result, understanding the methods of fruit waste transformation into valuable resources is crucial (Peng *et al.* 2019). The fermentation and acetification processes of fruits and other raw materials not consumed in fresh may be interesting means for the valorization of this vegetable biomass and may result in beverages and vinegars, for example. In fact, vinegars produced from by-products of food processing, fruit wastes, substandard fruit and agricultural surpluses

(Molelekoa *et al.*, 2018) is an attractive possibility of waste management with economical profits (Roda *et al.*, 2017).

To conclude, while some wastes are inevitable, the proper application of fruit wastes could contribute to improve environmental sustainability by minimizing the environmental impacts of these wastes and to improve food products with high nutritional values.

## **1.2. Vinegar**

### **1.2.1. History and definition of vinegars**

Based on a study by Boistean (2021), the word “vinegar” comes from the words “*vin*” and “*aigre*,” which literally means “sour wine” in French, and its production dates to 2000 BC. It's worth noting that vinegar was once thought to be a foodstuff by-product created when wine spoiled due to contact with air (Ho *et al.*, 2017a). According to Ho *et al.* (2017a), it has a history over 10,000 years, and, it always occurred at the same time with wine-making processes, and it is thought that ancient civilizations including Persians, Egyptians, and Mesopotamians discovered that wines turn sour when exposed to the air (Mazza and Murooka, 2009).

Vinegar is defined by FAO/WHO as any liquid suited for human consumption which is made entirely from appropriate items comprising starches or carbohydrates, or starch plus sugars, through a double fermentation process (alcoholic and acetous) (Sengun, 2019; Tanamool *et al.*, 2020). In the European Union, the established limits for residual ethanol content are strictly set, the residual alcohol content must not exceed 3 % by volume and the total acetic acid content must be at least 60 g/l (OJEU, 2015). In many civilizations across the world, vinegar has been applied as a flavoring or preservation agent in a variety of culinary products as well as a drink after diluting with water (Isham *et al.*, 2019). According to Bekatorou (2020), vinegar is also used to pickle, a process of preserving food by immersing it in vinegar and salt to keep it from deteriorating, a variety of foods, including fruits, vegetables and meats. They also mention that vinegar's strong acidity inhibits microbiological development, making it an excellent preservative and, at the same time, vinegar enhances the flavor of pickled foods (Bekatorou, 2020). Furthermore, because of their very low pH levels, vinegars have a wide range of non-food uses, including sanitizing and disinfection agents (Ramos *et al.*, 2014).

### 1.2.2. Biochemistry and substrate

Vinegar is produced by a two-stage fermentation procedure that begins with alcoholic fermentation of carbohydrates and ends with acetification (ethanol oxidation) (Bekatorou, 2020). Ho *et al.* (2017b) revealed that in anaerobic conditions, yeasts turn the fermentable carbohydrates in the substrate into alcohol, then, the alcohol is converted to acetic acid by acetous fermentation, which occurs in the presence of oxygen. Below is a summarized description of the two main types of fermentation methods used in vinegar manufacturing.

#### 1.2.2.1. Alcoholic fermentation

All vinegars go through a fermentation process known as alcoholic fermentation (AF). Microbes consume organic molecules in anaerobic circumstances to create energy (as ATP), carbon dioxide, and ethanol (Bekatorou, 2016). The activity of yeasts converts the fermentable carbohydrates into alcohol (Isham *et al.*, 2019). The alcoholic fermentation is mainly carried out by yeasts and *Saccharomyces cerevisiae* (Lynch *et al.*, 2019) because it is tolerant to high sugar and ethanol concentrations, low pH values, low temperatures, high pressures and sulfur dioxide (SO<sub>2</sub>) (Bekatorou, 2016; Jackson, 2008), although, other non-*Saccharomyces* species have been found during AF in vinegar production (Parapouli *et al.*, 2020). According to Bekatorou (2019), the AF is a relatively fast process and most accessible sugars are generally depleted within the first three weeks, which occurs via the Embden–Meyerhof–Parnas (glycolytic) route, that involves the conversion of hexose sugars like glucose into two molecules of pyruvate and two molecules of ATP per hexose molecule. Afterwards, the resulting pyruvate is decarboxylated into acetaldehyde and CO<sub>2</sub> by pyruvate decarboxylase enzyme (thiamine pyrophosphate coenzyme is required) and lastly, in a reaction involving oxidation of NADH to NAD<sup>+</sup>, the acetaldehyde gets reduced to alcohol-by-alcohol dehydrogenase enzyme (Bekatorou 2019; Carrascosa *et al.*, 2011).

#### *Saccharomyces cerevisiae*

The main genera of yeast involved in the alcoholic fermentation of fruits are *Saccharomyces*, and, as non-*Saccharomyces*, *Candida*, *Debaryomyces*, *Hanseniaspora* (and its anamorph *Kloeckera*), *Issatchenkia*, *Kluyveromyces/Lanchancea*, *Metschnikowia*, *Pichia*, *Torulasporea*, *Wickerhamomyces* (Parapouli *et al.*, 2020).

The genus of *Saccharomyces* is the most widely utilized genus associated to vinegar processing. Bekatorou (2016) and Matallana and Aranda (2016) revealed that *S. cerevisiae* possesses several distinguishing features that are not present in other genera, such as a greater ability in sugar fermentation, tolerance for elevated alcohol and sugar concentrations along with low temperatures and pH values, high pressures and sulfur dioxide. Bekatorou (2016) also mentioned to the fact that in the absence of oxygen, *S. cerevisiae* is able to fully convert carbohydrates to ethanol, generating modest levels of undesired chemicals (such as hydrogen sulfide, acetic acid, and urea) and cell mass.

Different yeast species overtake natural fermentations, like those used in traditional wine and vinegar production, relying on the medium structure, process parameters, and production phase (Bekatorou, 2016; Jackson, 2008).

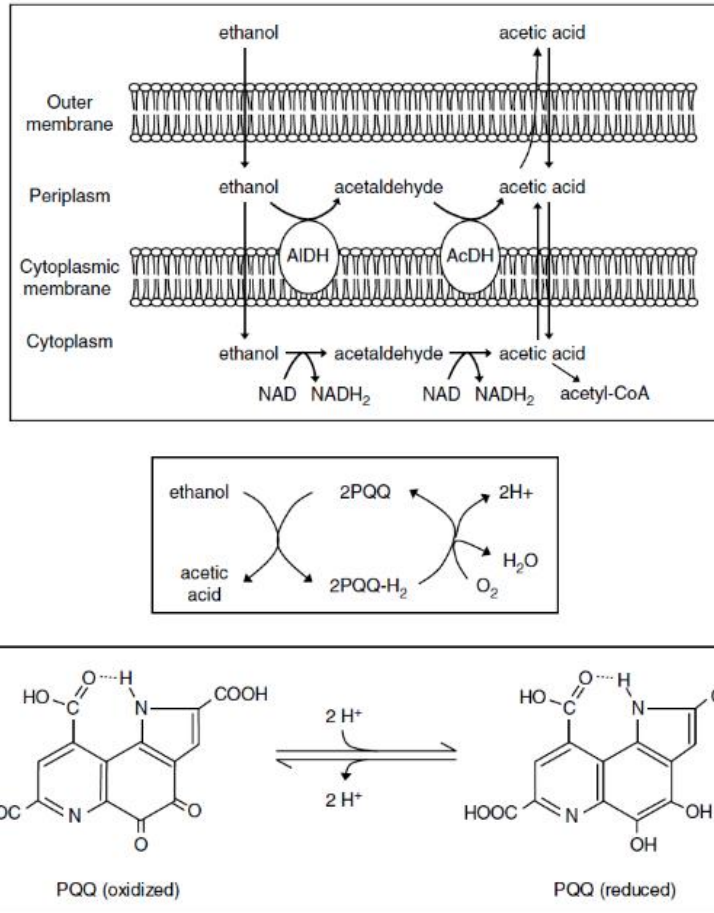
#### 1.2.2.2. **Acetification**

Acetic acid bacteria (AAB) are obligate aerobes Gram-negative bacteria with unique capacities to incompletely oxidize ethanol and carbohydrates into organic acids, perform acidification in vinegar manufacture and distribution (Kerstens *et al.*, 2006; Sengun, 2017 & 2020). According to Bekatorou (2020), membrane-bound *pyrroloquinoline quinone-dependent dehydrogenases* (*PQQ-dDs*), which are linked to the respiratory chain through the reduction of ubiquinone to ubiquinol, catalyze the incomplete oxidation. Reoxidation of ubiquinol is accompanied by oxygen reduction with *quinol oxidases*, as a result, when alcohols and sugars are oxidized into organic acids in the periplasm by *PQQ-dDs*, ATP is produced from oxidative phosphorylation (Bekatorou, 2020). Organic acids are stoichiometrically generated as dead-end metabolites that are not used as carbon sources within many cases, conversely, alcohols and sugars, such as ethanol and glucose, can be sometimes oxidized by the membrane-bound *PQQ-dDs* in the periplasm and, at the same time, the soluble *dehydrogenases* in the cytoplasm for both energy generation and carbon absorption (Arai *et al.*, 2016).

Organic substrates, in most oxidative processes (e.g., the Krebs or citric acid cycle), are normally oxidized to CO<sub>2</sub> and H<sub>2</sub>O, conversely, during vinegar production acetic acid bacteria generally oxidize the ethanol only to acetic acid (Bringer and Bott, 2016; Hutkins, 2019). A study by Gomes *et al.* (2018) showed that there are few cases when complete oxidation to CO<sub>2</sub> is possible.

As depicted in figure 1.2, there are just two major stages in the acetic acid synthesis. Ethanol is first oxidized to acetaldehyde, then, the acetaldehyde, is converted to acetic acid (figure 1.1) (Chen *et al.*, 2015; Saichana *et al.*, 2015). According to Hutkins (2019), there may possibly be an intermediary stage in which acetaldehyde hydrate is produced. In this process, oxygen acts as electron acceptor. It is worth noting that the transformation of ethanol into acetic acid proceeds on an equimolar basis, meaning that the ultimate concentration of acetic acid will be the same as the ethanol in the starting material once the reactions are finished (assuming negligible loss from evaporation) (Hutkins, 2019). Just a small portion of ethanol's carbon is utilized for biomass or transformed into other compounds (Hutkins, 2019).

Lastly, as stated above, the acetic acid route is thought to be an incomplete oxidation due to the fact that ethanol is partly oxidized, and the acetic acid produced is not fully oxidized. While certain acetic acid bacteria, including *Gluconobacter* can oxidize acetic acid, most *Acetobacter* species can oxidize it only in the suitable conditions (Bekatorou, 2020). The main requirement for acetic acid oxidation is the lack of ethanol in the media, since ethanol appears to inhibit the production of citric acid cycle enzymes (Mas *et al.*, 2014). According to Hutkins (2019), in real vinegar production process, acetic acid over oxidation is unfavorable since it results in the disappearing of the final product into the air. This is probably one of the reasons why vinegar fermentations have traditionally been done in a semi-continuous form, some quantity of ethanol available during the production process.



**Figure 1.1.** The formation of acetic acid from ethanol by AAB (Vidra and Németh, 2017).

## Acetic acid bacteria

Acetic acid bacteria (AAB) are Gram-negative aerobics with an ellipsoidal to rod like shape appearing in single form, in pair or in chain. Their ideal growth pH is about 5.0-6.5 and are able to grow at pH=3-4, with the optimum growth temperature about 28-30 °C (Mamlouk and Gullo, 2013; Qiu *et al.*, 2021). AAB may be found in a variety of sugar-rich plant materials including fruits, as well as alcohol-containing beverages such as wine, beer among others (Bekatorou, 2020; Qiu *et al.*, 2021; Trček and Barja, 2015).

AAB are found in the *Alphaproteobacteria* class, *Rhodospirillales* order, and *Acetobacteraceae* family (Kerstens *et al.*, 2006). There are numerous different varieties of AAB, the first of which, *Acetobacter*, was suggested and characterized by Beijerinck in 1898 (Beijerinck 1898). New genera and species have been discovered on a regular basis since the introduction of polyphasic

categorization techniques (Cleenwerck and De Vos, 2008), and 19 genera and 92 species of AAB have been reported to date (Qiu *et al.*, 2021).

The family of *Acetobacteraceae* is divided into 19 genera and 92 species including *Acetobacter* (29 species), *Acidomonas* (1 species), *Ameyamaea* (1 species), *Asaia* (8 species), *Bombella* (1 species), *Commensalibacter* (2 species), *Endobacter* (1 species), *Gluconobacter* (25 species), *Komagataeibacter* (15 species), *Kozakia* (1 species), *Neoasaia* (1 species), *Neokomagataea* (2 species), *Nguyenibacter* (1 species), *Saccharibacter* (1 species), *Swaminathania* (1 species), *Swingsia* (1 species) and *Tanticharoenia* (1 species) (Qiu *et al.*, 2021).

Some of the AAB, particularly *Acetobacter* and *Komagataeibacter*, are more efficient at converting alcohol to acetic acid than glucose. *Gluconobacter*, on the other hand, prefers to oxidize carbohydrate and sugar alcohol, than ethanol (Matsushita *et al.*, 2016).

*Acetobacter* and *Komagataeibacter* are principally responsible for the oxidation of ethanol to acetic acid in vinegar production, since both genera exhibit good resilience to high ethanol and acetic acid levels, which are significant features for industrial vinegar production (Taban & Saichana, 2017).

A variety of acetic acid bacteria (AAB) species have strains that can be utilized to make vinegar. *Acetobacter* genus includes species (e.g., *A. aceti*, *A. malorum*, *A. pasteurianus*) with strains capable of producing about 6 to 8 percentage of acetic acid; they are primarily necessitated in the production of vinegars with low-medium acetic acid content, conversely, *Komagataeibacter* genus includes species (e.g., *K. europaeus*) that are responsible for the production of vinegars with high acetic acid content (15-18 % acetic acid) (La China *et al.*, 2018; Sengun, 2017).

Although the bioconversion of ethanol to acetic acid in the vinegar production is classified as very efficient (approximately 90–95 percent of the stoichiometric yield) (Jiménez-Hornero *et al.* 2009), various evidences point to the necessity for further improvement (La China *et al.*, 2018).

It is worth noting that the kind of microbial culture employed in vinegar manufacturing is the main criteria that significantly differentiates vinegar production from the most other fermented foods and drinks (for example, sourdough for bread manufacture, dairy products, sausages, wine, and beer) (La China *et al.*, 2018). Particularly, based on several studies, most vinegar production is performed at both small and large scales by mixed microbial cultures of AAB without the use of

selected starting cultures (SSCs), and propagated by back-sloping procedure (Di Donna *et al.*, 2020; Gullo *et al.* 2014; Hidalgo *et al.* 2010; La China *et al.*, 2018; Mas *et al.* 2014). Di Donna *et al.* (2020) reported vinegar production performed via mixed AAB cultures, not only provides the execution of procedures with minimal technology needs and investments, but also restricts the full utilization of chosen strains' metabolic capabilities.

**Table 1. 1.** Physiological properties and characteristics of acetic acid bacteria (Yamada, 2016).

<b>Property</b>	<i>Acetobacter</i>	<i>Gluconobacter</i>	<i>Gluconoacetobacter</i>
<b>Temperature optimum</b>	25-30	25-30	25-30
<b>pH optimum</b>	5.4-6.3	5.5-6.0	5.4-6.3
<b>Acetic acid oxidation</b>	+	-	+/-
<b>Lactic acid oxidation</b>	+	-	+/-
<b>Acid from glucose</b>	+	+	+
<b>Ubiquinone type</b>	Q <sub>9</sub>	Q <sub>10</sub>	Q <sub>10</sub>

### **Factors affecting the acetous fermentation**

Factors that influence vinegar production can be defined in two different categories. The primary parameters are those related to the AAB metabolism and enzymatic activity, including pH, temperature and oxygen supply, while the secondary parameters are those that improve the acetic acid bacteria's metabolism activity and affect the acetification rates and the costs of the final products, such as design of fermenter, size of the operation and startup cultures (Tesfaye *et al.*, 2003). The mentioned parameters influence the entire acetification process and are described in detail below:

## **Oxygen supply**

Because of its solubility, oxygen is a limiting element in vinegar production, which is greatly influenced by the bioreactor structure, oxygenation system, temperature, and the chemistry of the substrate (Bekatorou, 2020). Acetification is a completely aerobic process in which a liter of ethanol takes approximately 400 liters of oxygen (Tefaye *et al.*, 2003). Ubeda *et al.* (2011) revealed only a minute delay in oxygenation can interrupt the acetification process, therefore, to guarantee optimal acetification processes, an appropriate amount of dissolved oxygen must be achieved and maintained, that will affect the rate and output of the fermentation, and the quality of the final product.

## **Temperature**

Generally, according to Bekatorou (2020), acetic acid bacteria are mesophilic with an optimal growth temperature of 25-30 °C, therefore, in the temperatures higher than this, they are deactivated because their key enzymes are denatured and their membranes are destroyed which results in the loss of cellular components, increase in the toxicity of the acetic acid and ethanol and finally, a loss of the volatile chemicals which in return, reduces both yield and quality of the final product. Because of the variety of the current species and the impact of the substrate composition, defining a range of temperature for these group of bacteria is challenging. Based on a study by Ho *et al.* (2017b), the AAB activity, in systems without temperature control (traditional processes), is preferred throughout the spring and summer.

The ideal operating temperature for industrial submerged systems is around 30 °C (Bekatorou, 2020). Since acetic acid fermentation is a thermodynamically advantageous aerobic process (approximately 8.4 MJ/L of ethanol oxidized), the temperature rises during the process, resulting in irreversible loss of AAB's metabolic activity; consequently, temperature management is required (Gullo *et al.*, 2014).

## **pH**

The ideal pH value for acetic acid bacteria growth is 5.5 to 6.3 (Raspur and Goranovic, 2008). Nevertheless, certain species may survive at lower pH values, such as in wine with a pH of 3.02

to 3.85 or in traditional balsamic vinegar processing, in which bacterial growth has been recorded at pH levels lower than 3 (Bekatorou, 2020; Ho *et al.*, 2017b). The resistance of AAB to low pH, significantly depends on several factors, including alcohol and oxygen concentrations, particularly, it diminishes at elevated ethanol and low oxygen levels (Gullo and Giudici, 2008).

## **Production method**

The employment of different processes, such as the Orléans, submerged, or generator methods, would impact the quality of vinegars. Dabija and Hatnean (2014) reported that the Orléans technique guarantees that the substrates are almost depleted to maximizing the ethanol and acetic acid concentration in the ultimate product, since it takes the longest time for producing vinegar in comparison to submerged and generator method.

### **1.2.3. The industrial process of vinegar production**

In order to production of vinegar, an ethanolic substrate must first be created, which can be made from several sources such as wine, cider, malt or rice mashes (Hutkins, 2019). Regardless of the source, the ethanol substrate is generally produced with the final product (vinegar). As a result, according to Hutkins (2019), the initial resources do not have to be of the greatest grade, however, it does not mean that low-quality ingredients should be utilized, though, as much of the final vinegar's taste and aroma qualities are generated from the starting material. Certainly, the vinegar will lack these taste and aroma qualities if distilled ethanol is employed as the substrate.

The second step of vinegar production, where the ethanol substrate is converted to acetic acid, can be accomplished in one of many ways, all of which rely on the rate of acetic acid bacteria performing oxidative fermentation of ethanol (Lynch *et al.*, 2019).

The open (or slow) method, the trickling generator process, and the submerged fermentation process are three technological strategies through which the vinegar may be obtained. Hutkins (2019) revealed that the open vat process and the trickling generator process are more generally employed, particularly by major vinegar producers, as they can run in a continuous mode and shorten fermentation time from weeks to days. Traditional open methods, on the other hand, can

be done in a semi-continuous way and are still used in many areas of the globe (Hutkins, 2019). Furthermore, the open vat method is the preferred method for superior vinegars, such as balsamic and other wine vinegars.

#### 1.2.3.1. **Open vat process (Orléans Method)**

The open vat process is based on acetic acid bacteria growing on the surface of vats, barrels, jars, or trays (Budak *et al.*, 2014). As it is simple to do and involves naturally occurring or wild cultures, this was certainly the first method for producing vinegar (Lynch *et al.*, 2019). If wine or other ethanol-containing materials, without any protection, being exposed to the air, they will most likely convert into vinegar. Despite the fact that there are numerous variants, barrel fermentations are representative of the European techniques that are still used today. Lynch *et al.* (2019) reported that the "Orléans" or "French" or "continuous" techniques (invented in France), is one of the earliest techniques for producing high-quality vinegars that are being used today. It's a sluggish and ongoing fermentation that takes place in huge oak barrels (> 200 liters) with a high diameter/height ratio (Bekatorou, 2020).

In the open vat process, AAB grows only at the surface and its growth is followed by formation of a polysaccharide-containing film or pellicle that synthesizes at the liquid-air interface, where the oxygen concentration is high (result from dissolved oxygen from air entering via holes in the barrel above the liquid's surface) and facilitate the conversion of ethanol to acetic acid (Hutkins, 2019; Lynch *et al.*, 2019; Ho *et al.*, 2017b; Sengun, 2015; Rogers *et al.*, 2013). However, this feature can be lost on occasion, particularly if cells are cultured under non-static environments. According to a study by Hutkins (2019), the produced film is essentially important for the success of the fermentation, and its disruption may delay the process, as the AAB must reform it before the fermentation continue. As a result, open vat process may take weeks to begin the fermentation and also several months to finalize it.

In 1886, the chemical structure of the pellicle/film was recognized as cellulose, a polysaccharide made by plants, but seldom by bacteria (Brown, 1886). In fact, various vinegar bacteria, namely *Acetobacter*, *Gluconacetobacter*, and the recently appointed genus, *Komagataeibacter*, are able to synthesize an extremely pure type of cellulose (Hutkins, 2019). As stated above, the bacteria develop the pellicle in the first place, which is as a result of several possibilities (Hutkins, 2019).

For instance, the film gives aerobic organisms a positioning advantage, as well as being safe from UV light by growing just below the coating. Furthermore, the pellicle plays as protector against predators and antibacterial agents with a large molecular weight.

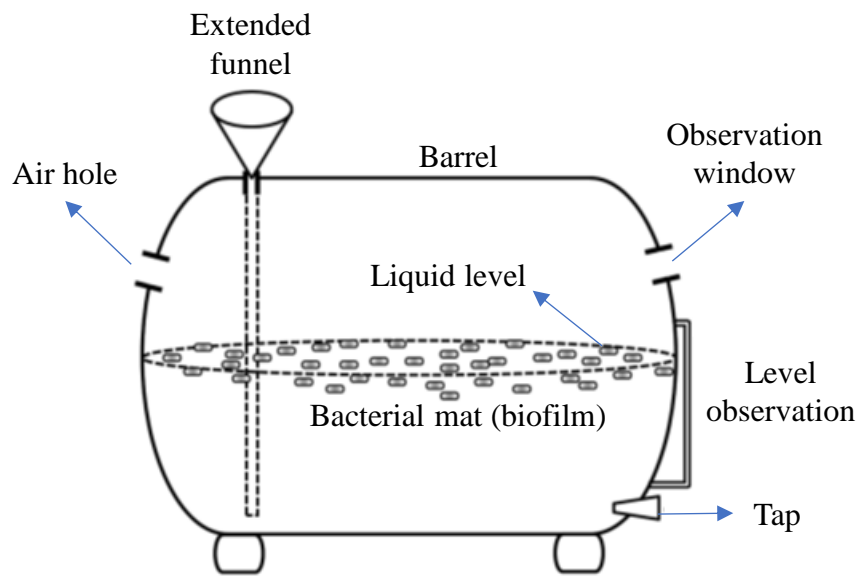
In the open vat process (Orléans method), the ethanolic substrate is placed in an appropriate vessel, and the fermentation is started either by naturally occurring acetic acid bacteria or “mother of vinegar”, an unknown starter culture obtained from the previous vinegar fermentation (i.e., backslopping) (Hutkins, 2019; Lynch *et al.*, 2019). The acetification is performed by a static culture of AAB that grow at the interface between the liquid and air (Sengun, 2015). The aeration, in the open vat process, is given via holes bored onto the sides of the barrel (covering with cheesecloth). In order to assist the ethanol oxidation and to leave an air chamber, barrels are filled to around 60-70% of their capacity and inoculated with a fresh vinegar culture, often known as "mother of vinegar" (Hutkins, 2019; Ho *et al.*, 2017a), while side holes allow circulation (Lynch *et al.*, 2019). At the bottom end, there is a tap that is used to extract the product. The culture develops a pellicle as previously said and starts oxidizing the ethanol. Once the acetic acid fermentation is complete, a part (between one-third and two-thirds) of the fermented vinegar can be discharged from the tap end (Hutkins, 2019; Mas *et al.*, 2014).

The Orléans method is often batch process, following fermentation completion, the barrel is emptied and subsequently is replaced with substrate (e.g., fresh wine). This step is performed without disturbing the film. However, the fermentation can be done in a semi-continuous mode, if steps are taken to keep the surface layer intact during the addition of substrate and removal of the product (Hutkins, 2019).

Depending on the initial composition of the substrate and alcoholic solution, the type of the existing microorganisms, the amount of oxygen provided, and the fermentation temperature, the procedure might last from 8-14 weeks for each full cycle (Hutkins, 2019; Dabija and Hatnean, 2014). Nowadays, vinegar producers utilize oak barrels and chosen or indigenous bacteria to make vinegar using the Orléans method, which takes place in total darkness at a fixed temperature (21–30°C) (Argyro Bekatorou, 2020). Vinegar is aged in wooden barrels under monitored circumstances for a minimum of 12 months and a maximum of several years (e.g., in cellars) (Bekatorou, 2020). However, aging process is usually reserved for high quality wine vinegars.

Despite the fact that alternative techniques take much less time, the quality of vinegar produced by the Orléans and equivalent barrel systems is considerably superior to that generated by faster methods.

According to Mas *et al.* (2014) the substrate raw material content and its preparation, the metabolic by-products of acetic acid bacteria during and after acetification, the substances extracted from the wooden barrels during ageing and the chemical and physical transformations that occur during the ageing process all contribute to the vinegar flavor. As a result, vinegars fermented by using slow Orléans method are known for their aroma complexity (soft and fruity) and are usually regarded as of excellent quality (Mas *et al.*, 2014). Furthermore, the process does not require heating, which could destroy the unique raw material aromas, and industries using this method typically do not use preservatives or pasteurize their vinegars, both of which are common industrial techniques that have been shown to degrade the delicate flavors of food and beverages (Bekatorou, 2020) (Figure 1.2).



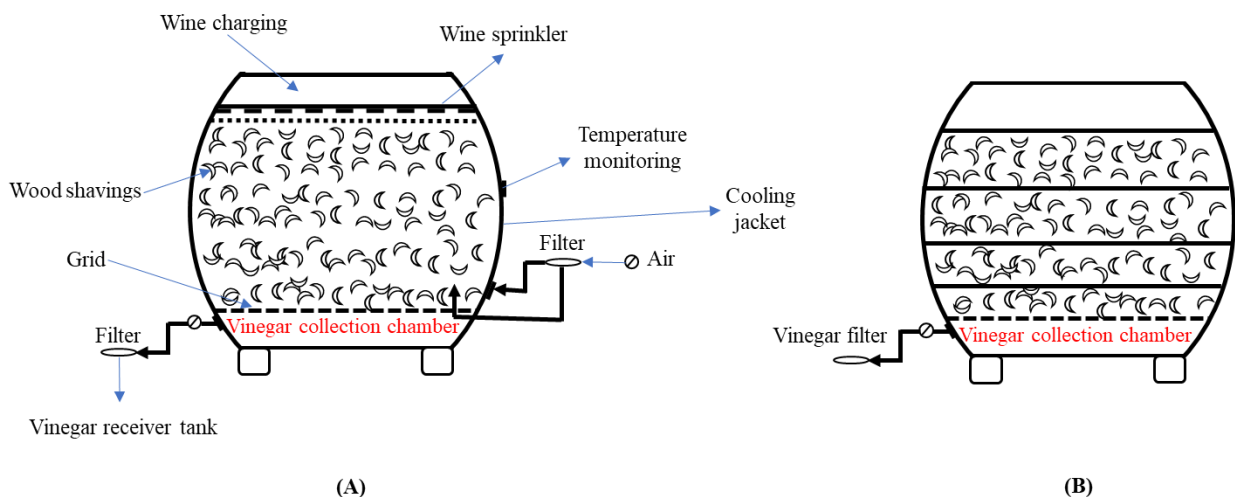
**Figure 1.2.** Slow ("Orléans") vinegar making in a barrel (adapted from Bekatorou, 2020)

### 1.2.3.2. The Quick/ Generator Method

During the vinegar fermentation, the rate of ethanol oxidation, in other words, the ability of acetic acid bacteria to perform the acetic acid fermentation, is dependent on the amount of oxygen present

and accessible, as well as the amount of surface area provided by the air-liquid interface. To put it another way, it is largely restricted by oxygen diffusion/transport from the atmosphere to the cell surface. Hence, it is probable to dramatically speed up the ethanol oxidation by expanding the surface area exposed to oxygen. The quick/ generator methods, also known as “Quick vinegar processes”, are based on this discovery, which was first observed about 300 years ago by the famed Dutch physician-chemist Hermann Boerhaave (Hutkins, 2019). The first example of the Quick method used on an industrial scale was by German scientists in 1823, notably Johann Sebastian Schützenbach, and have been used for over a century (Bekatorou, 2020).

The "Generator" or "German" techniques are enhanced vinegar manufacturing procedures in terms of speed and acetic acid output. In these systems ethanolic substrates are circulated or trickled through cylindrical fermentation vessels, which consists of a tall wooden (oak) or steel tank filled with materials soaked with vinegar such as curled wood shaving, wood staves, corn cobs, or stalks, which should not contribute off-flavors or are degraded (Lynch *et al.*, 2019) (figure 1.3). The main role of particulate packing materials is to maximize the total surface area inside the fermenter, particularly at the air-liquid interface, as inoculated substrate or feedstock moves around and between them (Hutkins, 2019). Acetic acid bacteria will then grow at the air-liquid interface, on the surface of the packing materials. Therefore, the ethanol concentration drops, and the acetic acid concentration rises during the passage of substrate from top to bottom (Hutkins, 2019).



**Figure 1.3.** Types of quick vinegar making vats with wood shavings (adapted from Bekatorou, 2020)

The main difference to Orléans method, in the production technique, is that in the quick fermentation the forced aeration is provided either via holes in the sidewalls or through the ventilated bottom of the generator, which also acts as a support for the particles (figure 1.2) (Bekatorou, 2020). When the liquid outflow reaches the bottom part (the collecting chamber), it can be recirculated to the top until it is acidic enough to be termed vinegar. A second tank, on the other hand, can be utilized to boost the oxidation rate and product production. The inoculated mash ferments initially in Tank 1, then is transferred back and forth between Tanks 1 and 2 with aeration until all the ethanol has been converted to vinegar. At the optimal temperature range of 27-30°C, full acetification takes around 3-7 days (Bekatorou, 2020; Hutkins, 2019). Released heat from oxidation can injure microorganisms, so it's important to keep the generator's temperature under control. Approximately two third of the generated vinegar is then taken from the bottom of the generator and replaced with wine in an equal proportion (Bekatorou, 2020).

These techniques generate greater acetic acid concentrations (up to 14 %) at higher rates, and the end products, which are usually regarded to be of acceptable quality, are obtained by appropriate water dilutions. The main strength of quick method compared to the traditional Orléans methods is that it requires less installation area and has less capital and operating costs. Conversely, evaporation may result in significant yield losses. Another issue is the need to refill the wood shavings on a yearly basis (Argyro Bekatorou, 2020).

#### 1.2.3.3. **The Modern Rapid Submerged Methods**

As early as the mid-1950s, the rapid submerged acetification methods have been utilized in industrial production of vinegar (Argyro Bekatorou, 2020; Tesfay *et al.*, 2002). While submerged fermentation systems are now widely applied for several non-foods industrial fermentation processes, they were invented by the vinegar business more than fifty years ago. Hutkins (2019) revealed that the Acetator (also known as the Frings fermenter), Cavitator, Bubble fermenter, as well as other similar elements are made of stainless steel, are easy to wash and sterilize, and may work in batch, semi-continuous, or continuous mode. The most successful and widely used submerged culture system is the Acetator, which is manufactured and sold by Heinrich Frings GmbH & Co. (Bonn, Germany) (Lynch *et al.*, 2019). Today, submerged fermentation methods are used to make the vast majority of vinegar produced around the world (Hutkins, 2019).

Fast and effective aeration is the most significant concept of the submerged fermentation systems (Lynch *et al.*, 2019). These methods involve forceful supply of air into an alcoholic mash contained in a suitable acetator tank, followed by acetification at about 26-30 °C (Tan, 2005).

According to Hutkins (2019), in addition to the aeration system, process controls for propellor speed (up to 1750 rpm), nutrient feed, foam-handling, and temperature further provide for consistent, continuous operation. since ethanol oxidation is an exothermic reaction in which heat is released, temperature control may be important, notably in large-scale fermentations. They also revealed that the medium temperature, unless monitored, can rise from an ideal range of 28-30 °C to as high as 33 °C, which may lead fermentation output to drop dramatically (Hutkins, 2019).

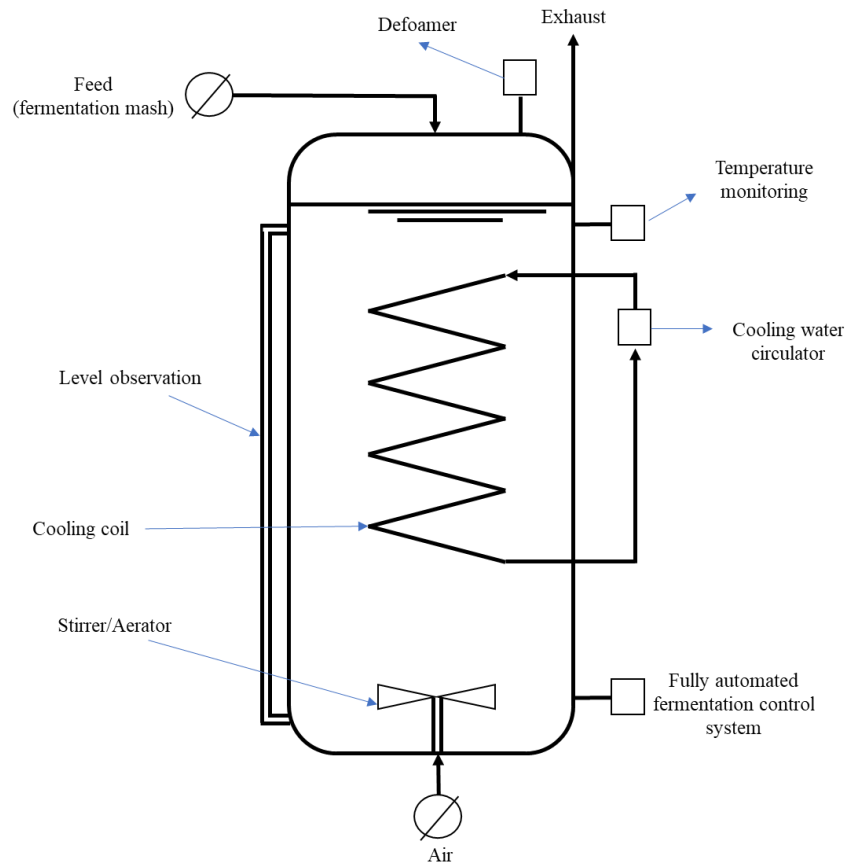
Generally, the initial material consists of both ethanol and acetic acid (from a previous fermentation). Hutkins (2019) revealed that the ethanol concentration will drop from approximately 5% to < 0.5%, during a single cycle of 16-24 hours, while the acetic acid level will rise from around 7% to 12%. Each batch is finished with roughly a third of the fermented liquid being drained away, and the tank is refilled with fresh ethanol feedstock to initiate the process for a second acetification batch. During a normal fermentation cycle, cell development is very slow, with only a single doubling of the starting population (Hutkins, 2019). The acetators are huge stainless-steel tanks with air pumps at the base that deliver air to the tank (figure 1.4). The acetators are generally equipped with nutrient input, heating, and tracking tools. After each outflow, the generated vinegar can be fed through a sequence of clarification, filtration, stabilization, and packaging machines (Bekatorou, 2020).

The main differences between the current rapid submerged procedures and the traditional method, are the process's speed (vinegar production in 24-hour cycles) and high acidity (acetic acid levels of around 23-25 %, as opposed to 6-13 % with typical methods) (Lynch *et al.*, 2019). Higher acidity reduces water moves, which, as a result, makes transportation more economical. The greater the ultimate acidity desired, the more precise process control is needed (Frings, 2018).

During the manufacturing of industrial vinegar, the key variable that must be regulated is the amount of alcohol present (Bekatorou, 2020). As a result, alcohol monitoring systems are critical components of modern commercial submerged vinegar manufacturing processes, determining fermentation rates and discharge times. According to Bekatorou (2020), submerged systems are

more suitable for high-strength processes (>17 percent acidity) which require continuous overview and ensure process control.

Programmable logic control systems (PLCs) are now the main process control systems, assessing all data collected by monitoring systems to guarantee cost-effective production, low product failure, and great quality. As a last point, many acetators situated anywhere in the globe can be linked to one central control room for communication (Frings, 2018).



**Figure 1.4.** A modern submerged fermentation acetator (based on the Frings technology) (adapted from Bekatorou, 2020).

#### 1.2.4. Vinegar quality

Since vinegars are not necessarily created from wine and can be made from grape, beer and apple cider, it is necessary to characterize different parameters for quality and classification. First of all,

since each nation has different rules or legislation regarding vinegar, there are numerous analysis criteria that may be used to describe a vinegar.

Hutkins (2019) revealed that, unlike other fermented foods, which their originality is rarely questioned, vinegar may be tampered, either with cheaper vinegar or with other acidic substances. Adulteration can also be caused by even more dubious methods. As instance, chemically synthesized acetic acid could be diluted and fraudulently sold as fermentation derived vinegar (Hutkins, 2019). As a result, the initial quality criterion is focused on confirming that the vinegar is vinegar (rather than diluted acetic acid) and that the kind of vinegar listed on the package correctly reflects what is in the product. Accomplishing this criterion, on the other hand, is a difficult task that necessitates extensive testing.

Acidity, residual alcohol and the acetic acid/ethanol ratios are the most frequent and conventional analytic criteria used to characterize vinegars (Solieri and Giudici, 2009). Nevertheless, the overall acidity level of vinegar differs from one vinegar to other, and from one region to the other. As a result, the Food and Drug Administration (FDA) mandates that any product labeled vinegar, should contain at least 4% of acid. Because the acid content of the products varies depending on several factors, the defined limitations for acidity and residual ethanol concentration in the European Union are closely enforced. As a result, wine vinegar's acidity (acetification produced purely from wine) must be at least 6% (w/v), with a maximum residual ethanol of 1.5% (v/v) (Mas *et al.*, 2014; Ji-Yong *et al.*, 2013). The amount of acetic acid and ethanol produced varies, as stated above, depending on the raw materials utilized, the microorganisms used in the fermentation, and the technique employed (Argyro Bekatorou, 2020). However, they differ widely depending on the vinegar type, so the acidity level isn't truly a quality indicator.

Generally, the nutritional value, safety, and sensory characteristics of food are the factors that determine its quality. However, since vinegar is mostly used as a condiment, sensory qualities play an important role in determining its quality, and the sensory quality of vinegar is chiefly affected by its aroma and flavor (Argyro Bekatorou, 2020).

The acetic acid fermentation process is primarily responsible for vinegar's distinct flavor and aroma (Ho *et al.*, 2017a). The raw ethanolic substance from which vinegar is produced has a significant impact on its flavor; it contains a variety of quality-related components, such as volatile aromatic compounds and polyphenols (Argyro Bekatorou, 2020; Ozturk *et al.*, 2015). Hutkins

(2019) conducted those other alcohols and aldehydes are oxidized by alcohol dehydrogenase and aldehyde dehydrogenase enzymes, which oxidize ethanol and acetaldehyde, respectively. If these substrates are present in the must, mash, or other starting material, a wide range of acidic and nonacidic final products can be generated. Formic, propionic, and butyric acids, as well as glycerol, are among these compounds. Diacetyl, acetoin, and ethyl acetate are some of the other volatile chemicals present in vinegar (Hutkins, 2019). Ozturk *et al.* (2015) claimed that organic acids, volatile compounds and other fermentation products are notably effective on the product's organoleptic characteristics (color, flavor, and astringency) along with its beneficial aspects.

Undoubtedly, the variety of bacteria engaged in acetification has been shown to have an impact on the vinegar's ultimate composition (Tesfaye *et al.*, 2002b). Additionally, the acetification method applied for vinegar manufacturing has a significant impact on the final aroma quality (Argyro Bekatorou, 2020).

Ageing in wood contributes to the increase in the aroma complexity of vinegars, and it also affects the vinegar color, which are both important aspects for costumers to evaluate the quality of a food product (Argyro Bekatorou, 2020). The time and type of ageing (in various types of woods) are other causes of variations and significantly influence vinegar quality (Callejon *et al.*, 2010; Ríos-Reina *et al.*, 2017).

Eventually, despite vinegar type, the existence of extracts, sugars, colors, artificial colors, or preservatives in a vinegar should also be taken into account with respect to the quality, since their existence can commonly be evident of a lower-quality vinegar (Bekatorou, 2020).

Also, several research focus on the categorization and authentication of vinegars, which is particularly important for the brand protection of high-quality PDO (Protected Designation of Origin) vinegars and the anti-adulteration and anti-competitive practices (Ríos-Reina *et al.*, 2017 & 2018). For this aim, spectroscopic and chemometric technologies, as well as their combinations, are provided.

### **1.2.5. Bioactivities and Health Benefits of Vinegar**

Vinegar has traditionally been used for its health advantages, which are scientifically verified. The antioxidative, antibacterial, anticancer, anti-obesity, antihypertensive, and cholesterol-regulating

properties of vinegar are due to the presence of bioactive components such as acetic acid, gallic acid, catechin, epicatechin, chlorogenic acid, caffeic acid, p-coumaric acid, and ferulic acid (Budak *et al.*, 2014).

Mas *et al.* (2014) reported that rather of preventing disease, several bioactive components have been found to be able to affect the risk of disease.

The phrase "food bioactive component" refers to non-essential biomolecules that are found in foods and have the capacity to influence one or more metabolic processes, resulting in the promotion of improved health, according to an entry in the *Encyclopedia of Food and Culture* (Katz and Weaver, 2003).

Bioactive compounds, similarly to the preceding definition, are:

- According to Nahler (2013), extra-nutritional or non-nutritional compounds of food that can have beneficial health effects; Generally, this excludes the vital nutrients.
- Additional nutritional components that are found in low quantities in foods. They are intensively researched in order to determine their health impacts (Kris-Etherton *et al.*, 2002).
- Non-essential natural substances found in, or produced from, plants, animals, or marine sources that are capable to alter biochemical, physiological, and metabolic processes in the human body while providing benefits beyond fundamental nutritional capabilities (Biesalski *et al.*, 2009; Liu, 2003).
- Gry *et al.* (2007) revealed that, non-nutritive components of food plants that promote health benefits and/or have damaging effects when consumed.

Yahia (2017), suggested that bioactive compounds are non-essential (non-nutritional), since one substance (or molecule) cannot have two physiological roles in the same organism; one nutritional (energetic metabolism and development) and the other bioactive. This suggestion attempts to differentiate between two processes: one that necessitates the degradation of the compound or molecule in order to release the energy required for the organism's functioning and development, and the other that demands the interaction of the compound (or molecule) in its environment with one or more living substances (Guaâdaoui *et al.*, 2014). Thus, bioactive compounds are classified

as substances that are able to interact with one or more components of live tissue, possibly causing a wide range of consequences (Guaâdaoui, 2014).

Polyphenols are the most common bioactive compounds found in in plant-based foods like fruits, vegetables, cereals, chocolate, herbs, dry legumes, and beverages such as fruit juices, tea, coffee, and vinegar, making them a great supplier of bioactive compounds in the diet (Denny and Buttriss, 2007; Yahia, 2017).

Polyphenols, chemically, are a large heterogeneous group of bioactive compounds marked by hydroxylated phenyl moieties. Based on the number of phenol rings present in their structure, as well as the binding elements between phenolic rings, these bioactive compounds can be classified into different groups, including flavonoids (e.g., catechin, quercetin, epicatechin), phenolic acids (e.g., gallic, ferulic, ellagic), stilbenes (e.g., resveratrol), and lignans (e.g., pinoresinol, podophyllotoxin, steganacin) (Scalbert *et al.*, 2005).

Due to polyphenols health benefits in humans after absorption, such as the avoidance of cardiovascular illnesses and cancer (Scalbert *et al.*, 2005), they have sparked a lot of attention. Antioxidant, anti-inflammatory, and anticarcinogenic qualities are credited with these health benefits (Daglia, 2012; Yahia, 2010).

Polyphenolic compounds, in addition to their health benefits, are also responsible for the color and astringency of vinegar, have sparked a lot of attention in this regard (Mas *et al.*, 2014).

Furthermore, vinegar comprises polyphenols, which have been proved to protect against lipid peroxidation, hypertension, hyperlipidemia, inflammation, DNA damage, and cancer (Chou *et al.*, 2015; Osada *et al.*, 2006; Pandey and Rizvi, 2009; Prior and Cao, 2000). Although acetic acid is the major ingredient, vinegars also include a variety of bioactive components that, depending on the raw materials and production processes employed, can have a variety of impacts on human health (Bekatorou, 2020).

Vinegar is commonly used as an acidic condiment, to pickle fruits and vegetables, and to make mayonnaise, salad dressings, and other food flavors. Furthermore, vinegars have long been employed as a food preservative due to their capacity to inhibit microbial growth (Saha and Banerjee, 2013; Tan, 2005).

According to Fushimi *et al.* (2006), taking 0.3% of dietary acetic acid may help lower cholesterol and triglyceride levels in the blood. It was shown that acetic acid bacteria generate alkali-stable lipids (ASL), which have a substantial effect on boosting cognitive capacity because they include extremely pure free dihydroceramide, a precursor to several sphingolipids, including the neurotransmitter gangliosides (Fukami *et al.* 2010).

### 1.3.Objectives

The overall objective of this work was to study the characteristics of four different fruit vinegars (Fig enxario, Fig, Sweet potato and Grape) from Algarve. This work also aimed to valorize non-marketed fruits. The specific objectives of the study were identified as:

1. To evaluate the physico-chemical properties (pH, Total acidity, °Brix) of the vinegars
2. To determine the bioactive properties of the vinegars (namely, Fig enxario vinegar, Fig vinegar, Sweet potato vinegar and Grape vinegar)
3. To measure the color parameters (L\*, a\* and b\*) of the vinegars
4. To evaluate the microbial quality of the vinegars through the enumeration of total microbial counts, lactic-acid bacteria, acetic-acid bacteria and filamentous fungi and fermentative yeasts
5. To assess the antimicrobial activity of the vinegars on different microorganisms (Gram-positive (*Staphylococcus aureus* ATCC 25923, *Listeria innocua* ATCC 33090, *Staphylococcus epidermidis* ATCC 12228 and *Bacillus cereus* ATCC 10876) and Gram-negative (*Cronobacter sakazakii* ATCC 29544, *Salmonella enterica* Typhimurium ATCC 14028, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) bacteria and yeasts (*Candida albicans* ATCC 10231, *Candida albicans* ATCC 90028, *Cryptococcus neoformans* YPO 186, *Saccharomyces cerevisiae* PYCC 3507, *Pichia membranifaciens* PYCC 2489, *Zygosaccharomyces bailii* PYCC 4806 and *Debaryomyces hansenii* PYCC 2968)).

## **2. Materials and methods**

## 2.1. Sample collection

Four different vinegar samples were collected (in sextuple) from a vinegar production factory, Fateixa, located in Algarve, Portugal. The vinegars are commercialized in bottles of 250 ml. These vinegar samples consisted of Fig enxario vinegar, Fig vinegar, Sweet potato vinegar and Grape vinegar with acetic acid concentrations of 4.8%, 5.8%, 6.4% and 6.5%, respectively (figure 2.1).



Figure 2.1. Commercialized vinegar samples used in this study

## 2.2. Physico-chemical characterization

### 2.2.1. pH measurement

The pH measurement was taken using a pH-Meter (Crison, Model Basic 20, Barcelona, Spain) with a glass electrode (with an automatic paired temperature reading) calibrated with buffer solutions to pH 4.0 and 7.0 (figure 2.2). Measurements were taken in duplicate and mean values were reported (Akbas & Cabaroglu, 2010; Ozturk *et al.*, 2015; Bakir *et al.*, 2017).



**Figure 2.2.** pH-Meter (Crison, Model Basic 20, Barcelona, Spain)

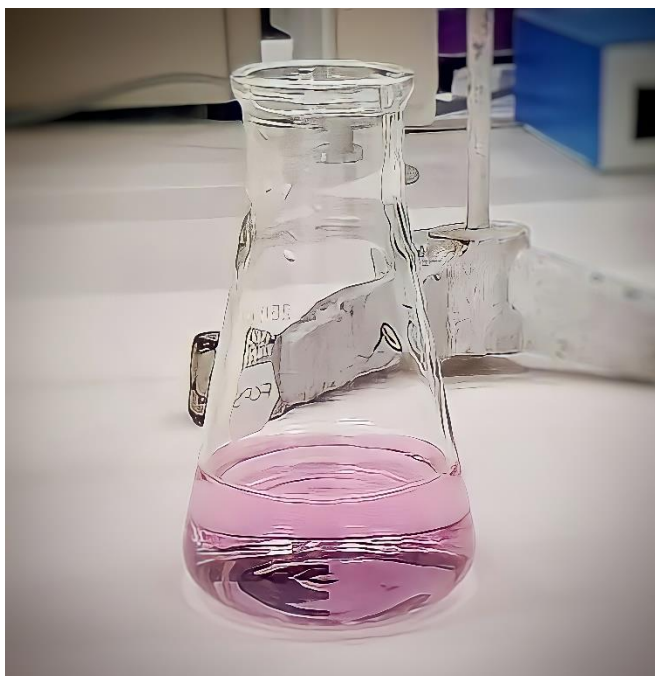
### 2.2.2. Acetic acid measurement

Titrateable acidity (TA) was determined as ml of 0.1 M sodium hydroxide (NaOH) (Rhône-Poulenc, Paris, France) used to obtain a pink color endpoint in the presence of Phenolphthalein 2% (Merck, Germany) (figure 2.3). Acetic acid is the major organic acid in vinegar. The titration acidity as percent was calculated using the following equations (eq. 1 and eq. 2): (Bakir *et al.*, 2017)

$$\text{Acidity index (g/L)} = \frac{V_2 \times M \times 60}{V_1} \quad (\text{equation 1})$$

$$\% \text{ acidity} = \frac{A.I.}{\text{Density} \times 10} \quad (\text{equation 2})$$

where  $V_1$  is the volume of the vinegar sample,  $V_2$  is the volume of the NaOH spent on sample titration and  $M$  is the molarity of the titrant solution. Experiments were conducted in triplicate and mean values were reported.



**Figure 2.3.** Endpoint color in acetic acid measurement using titration method

### **2.2.3. Determination of total soluble solids (°Brix)**

Brix values of the vinegars were measured using Abbe refractometer (Atago, 1T, Japan) (figure 2.4) calibrated with distilled water. The values were expressed as °Brix (Akbas & Cabaroglu, 2010; Ozturk *et al.*, 2015). Measurements were taken in duplicate and mean values were reported.

### **2.2.4. Color measurement**

Color values of the vinegars were measured using a handheld colorimeter and color was represented by the L\* a\* b\* color notation. This is a 3-D color presentation method in which L\* is the lightness of color and equal to 0 for black and 100 for white, a\* is the amount of red (0 to 60) or green (0 to -60) whereas b\* is the amount of yellow (0 to 60) or blue (0 to -60) (Mallikarjunan and Mittal, 1994). The equipment was first calibrated with a white pad and black background. Samples were presented on sample holder. Means of five measurements were taken at different portions of the sample were recorded.



**Figure 2.4.** Abbe refractometer (Atago, 1T, Japan)

### **2.2.5. Determination of total phenolic content (TPC)**

The total content of the phenolic compounds was analyzed by applying the Folin–Ciocalteu method (Zou *et al.* 2014) with some modifications and using gallic acid 98% (Acros Organics, Geel, Belgium) as the standards (0.00, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 mg/ml of gallic acid). A standard calibration curve was obtained (equation 3).

In brief, the appropriate dilution of vinegar (0.2 ml) was combined with the 1 ml of 0.2 N Folin–Ciocalteu reagent (Merck, Germany). Then, 0.8 milliliter of 7.5% (m/v) sodium carbonate were added to the mixture and after the proper shaking, the solution was allowed to stand at room temperature in the dark for 30 minutes. The absorbance of the solution at 765 nm was measured with a spectrophotometer (Spectronic GENESYS 10) (figure 2.5) and the result were expressed in mg of gallic acid equivalent (GAE) per milliliter (mg GAE/ml).

The total content of the phenolic compounds was calculated using the following equation 3 based on the calibration curve:

$$A = 7.6752 C + 0.0189 \quad R^2 = 0.9937 \quad (\text{Equation 3})$$

where A is the absorbance and C is the total phenolic content in mg/ml. Experiments were conducted in duplicate and mean values were reported.

### 2.2.6. Determination of total flavonoid content (TFC)

Total flavonoid content was determined according to Dewanto *et al.* (2002). Briefly, 1 ml of the vinegar sample was added to 4 ml of distilled water in a test tube followed by addition of 0.3 ml of a 5% NaNO<sub>2</sub> solution and vigorously mixed with vortex. The mixture was kept in the dark at room temperature. After 5 min, 0.3 ml of a 2% Aluminum chloride (AlCl<sub>3</sub>) (Riedel-de Haën, Germany) was added, shaken using vortex, and allowed to stand for 6 minutes in the dark.

After, 2 ml of 1 M NaOH (Riedel-de Haën, Germany) was added followed by the addition of 2.4 ml of distilled water and mixed well. The absorbance was measured immediately against the blank at 510 nm wavelength using a spectrophotometer (Spectronic GENESYS 10) (figure 2.5) in comparison with the standards prepared similarly with (+)-catechin (Sigma-Aldrich, United States) concentrations of 0, 10, 50, 100, 200, 300, 400 and 500 mg/ml. The flavonoid content was calculated using the following linear equation based on the calibration curve (equation 4):

$$A = 0.0022 C + 0.0079 \quad R^2 = 0.9905 \text{ (Equation 4)}$$

where A is the absorbance and C is the flavonoid content in mg/ml. Total flavonoid contents of vinegar samples were expressed as milligrams of catechin equivalents per milliliter of vinegar (mg catechin/ml). Experiments were conducted in duplicate, and values were reported as mean and  $\pm$  SD.

### 2.2.7. Determination of antioxidant capacity (TAC)

The antioxidant activity of the vinegars, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca *et al.* (2001). The vinegar samples (0.1 ml) were mixed with 5 ml of a 0.1 mM DPPH (Sigma-Aldrich, United States) solution in test tubes vigorously mixed with vortex. Methanol (0.1 ml) in place of the vinegar sample was used as control. Following the incubation for 13 minutes, in the bath at 27°C in the absence of light, absorbance values were measured at 515nm wavelength, against methanol (MeOH), using a spectrophotometer (Spectronic GENESYS 10) (figure 2.5).



**Figure 2.5.** Spectrophotometer (Spectronic GENESYS 10)

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) ( $C_{14}H_{18}O_4$ ) (Sigma-Aldrich, United States) with the concentration of 0.08, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35 and 0.40 mg/ml, was used for generating a standard curve. The total antioxidant capacity of the vinegar samples was calculated using the equation 5 based on the calibration curve:

$$A = -0.4504 C + 0.5635 \quad R^2 = 0.9948 \quad (\text{Equation 5})$$

where A is the absorbance and C is the total antioxidant capacity in mg/ml. The antioxidant activity of the vinegar samples was expressed as mg of Trolox equivalents per milliliter (mg TE/ml). Experiments were conducted in triplicates and mean values were reported as mean and  $\pm$  SD.

## **2.3. Microbiological characterization**

### **2.3.1. Microbial quality evaluation**

The microbial quality assessment of the vinegars was performed through the study of the following parameters: mesophilic aerobic microorganisms, lactic acid bacteria, filamentous fungi, fermentative yeasts and acetic acid bacteria.

### **2.3.1.1. Dilution preparation**

In this regard, three samples (bottles) of each vinegar were mixed properly to obtain a homogenized solution and consequently, different dilutions of the vinegar were prepared. Afterwards, 10 ml of each vinegar was pipetted into 90 ml (1:10 dilution) of sterile Ringer solution (Oxoid, U.K.) in a sterilized flask. The mixtures were homogenized with a vortex mixer (VWR, U.S.A.). Subsequently, two more dilution steps were performed ( $10^{-2}$  and  $10^{-3}$ ).

### **2.3.1.2. Enumeration of mesophilic aerobic microorganisms**

To enumerate the total mesophilic aerobic microorganisms the medium “Plate Count Agar” (PCA, Scharlau, Spain) was prepared according to the manufacturer’s instructions in two separate flasks. The pH values of the media were adjusted to 5.5 and then they were autoclaved at 121 °C for 15 minutes. In the next step, 15-20 ml of the media was poured into each plate and then were allowed to solidify. Next, 0.2 ml of each vinegar and its serial dilutions were inoculated on the plates with PCA and were spread evenly across the entire surface of the solid media. Each vinegar/dilution were analyzed in duplicate. Thereafter, the plates were incubated at 30 °C for 72-96 hours. Colony counting was performed after the incubation period. The results obtained were expressed in CFU/ml. The counting of mesophilic aerobic microorganisms was performed according to the ISO 4833-1 (2013), with some modifications.

### **2.3.1.3. Enumeration of total viable acetic acid bacteria (AAB)**

In order to enumerate the total viable acetic acid bacteria (AAB) present in the vinegars, “AAB-selective agar” (ABS) was used (Kim *et al.*, 2019).

ABS was prepared as described by Kim *et al.* (2019). The ingredients D-(+) Glucose 50 g (Scharlau, Spain), yeast extract 10 g (BIOKAR Diagnostics, Adana, Turkey), bromophenol blue 20 mg (BDH, Dubai, United Arab Emirates), and bacteriological agar 20 g were dissolved in 950 ml of distilled water and autoclaved at 121 °C for 15 min. After cooling in a water bath at 50 °C (figure 2.6.), 1 ml of glacial acetic acid, 50 ml of ethanol 96% v/v (Panreac, Spain) were added to the media. The pH of the media was adjusted to 4.8 at 25 °C. After thorough mixing, 15-20 ml of the media was poured into each plate.

After the solidification of the medium, 0.2 ml of each vinegar and its serial dilutions were inoculated on the surface of the plates with ABS and were spread evenly over the entire surface of the media. Each vinegar/dilution were analyzed in duplicate. The prepared plates were incubated at 30 °C for 72-96 hours. AAB colonies can be easily observed and differentiated on ABS as they form green colonies with yellow halos surrounding them (Kim *et al.*, 2019). Bromophenol blue, a pH indicator, is crucial for the development of the green-to-yellow colony color induced by acid-producing microorganisms (Flores, 1978). Followingly, colony counts were performed 72-96 hours after the inoculation. The results obtained were expressed in CFU/ml.

#### **2.3.1.4. Enumeration of Lactic acid bacteria (LAB)**

Experiments were conducted to enumerate the Lactic acid bacteria (LAB) (microaerophilic microorganisms) using “Man Rogosa and Sharpe Agar” (MRS, Scharlau, Spain). In this regard, the MRS media was prepared, according to the manufacturer’s instructions, and after the autoclaving at 121 °C for 15 minutes, the media was allowed to cool in a water bath at 50 °C. Afterwards, 1 ml of each vinegar and its decimal dilutions (in duplicates) were pipetted and transferred into the empty plates and then, the previously prepared MRS was added to the plates. Plates were allowed to get solid and thereafter, a second layer of the MRS was added on the previous layer. After the solidification of the second layer, the plates were incubated at 30 °C for 3-4 days. Colony counts were performed 72-96 hours after the incubation. The results obtained were expressed in CFU/ml. The counting of lactic acid bacteria was performed according to the ISO 15214, British Standard (1998).

#### **2.3.1.5. Enumeration of fungi**

With the aim of filamentous fungi enumeration, “Dichloran Rose Bengal Chloramphenicol” media (DRBC, Scharlau, Spain) was prepared, according to the manufacturer’s instructions, and autoclaved at 121 °C for 15 minutes. Then, the medium was dispensed into the plates. After the solidification, 0.2 ml of each vinegar and its serial dilutions were inoculated on the plates and were spread evenly on the surface of the media. Each vinegar/dilution were analyzed in duplicate. The

plates were incubated at 25 °C for 5 days. The counting of filamentous fungi was performed according to the ISO 21527-1 (2008).



**Figure 2.6.** Cooling down the autoclaved media in water bath with temperature adjustment at 50 °C.

#### **2.3.1.6. Enumeration of yeasts**

The enumeration of fermentative yeasts was performed in “Malt Extract Agar” (MEA, Scharlau, Spain) (Santo *et al.*, 2012) which was prepared, according to the manufacturer’s instructions, and after the autoclaving at 121 °C for 15 minutes. The pH of the medium was adjusted to 5.5 at 25 °C. The plates were inoculated by surface plate technique as described previously. Each sample/dilution were analyzed in duplicate. Finally, the plates were incubated at 25 °C for 5 days. Colony counts were performed on the 3<sup>rd</sup> and 5<sup>th</sup> days after the incubation. The results obtained were expressed in CFU/ml.

### **2.3.2. Antimicrobial activity**

The antimicrobial activity of vinegars was evaluated using the Kirby-Bauer method, also known as agar disc diffusion, according to the antimicrobial susceptibility testing EUCAST disk diffusion method, described by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) with slight modifications (EUCAST, 2020).

#### **2.3.2.1. Microbial sample collection**

The antimicrobial properties of the vinegars (Fig enxario vinegar, Fig vinegar, Sweet potato vinegar and Grape vinegar with acetic acid concentrations of 4.8%, 5.8%, 6.4% and 6.5%, respectively) were evaluated against 15 microorganisms (Gram-positive bacteria including *Staphylococcus aureus* ATCC 25923, *Listeria innocua* ATCC 33090, *Staphylococcus epidermidis* ATCC 12228 and *Bacillus cereus* ATCC 10876 and Gram-negative bacteria including *Cronobacter sakazakii* ATCC 29544, *Salmonella enterica* Typhimurium ATCC 14028, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, and fungi strains including *Candida albicans* ATCC 10231, *Candida albicans* ATCC 90028, *Cryptococcus neoformans* YPO 186, *Saccharomyces cerevisiae* PYCC 3507, *Pichia membranifaciens* PYCC 2489, *Zygosaccharomyces bailii* PYCC 4806 and *Debaryomyces hansenii* PYCC 2968).

#### **2.3.2.2. Antimicrobial activity assessment experiments**

Bacterial samples were cultivated on plates with Mueller Hinton agar (MHA, Himedia) and yeasts were cultivated on YM (YM Agar, Himedia) or MHA supplemented with 2% glucose (w/v) in the case of the yeasts of the species *Candida albicans*. Plates were incubated at 30 °C for 24 h (for bacteria) and 48 h (for yeasts). Thereafter, microorganism suspensions were prepared individually by emulsifying an appropriate number of the colonies into the 5 ml of sterile saline solution (NaCl 0.85%) and adjusting their turbidity to the McFarland scale 0.5 [ $\sim 10^6$  colony forming units (CFU) per ml] turbidity standard.

The effect of the antimicrobial activity of the vinegars was evaluated on Mueller Hinton agar (MHA, Himedia) for bacterial tests and on YM medium (YM Agar, Himedia) for yeasts experiments. In the experiments conducted with *Candida albicans* ATCC 10231 and *Candida*

*albicans* ATCC 90028, the strains were cultured in Mueller Hinton agar (MHA, Himedia) supplemented with 2% glucose (w/v). The pH of the all the media used in these experiments was adjusted to 5.5. Using sterile swabs, the bacteria and yeast suspensions were inoculated on the surface of the media in order to obtain a confluent and even microbial growth across the entire plate surfaces. Then, the inoculated plates were allowed to dry for 5 minutes. Subsequently, sterile filter paper blank discs (6 mm in diameter, Liofilchem, Italy), were placed onto the surface of the agar plates previously inoculated with the microorganisms' standard suspensions to be tested.

Each disc was impregnated with 20  $\mu$ L of the vinegars. As negative control 20  $\mu$ L of 0.85% saline solution was used in a disc.

Positive controls in this study for bacterial assessments were Ampicillin (10  $\mu$ g, Oxoid, United Kingdom), Penicillin G (10 IU/disc, Liofilchem, Italy), Chloramphenicol (30  $\mu$ g/disc, Liofilchem, Italy) and Erythromycin (15  $\mu$ g/disc, Liofilchem, Italy). In the case of yeasts, positive controls were Fluconazole (25  $\mu$ g/disc, Liofilchem, Italy), Amphotericin B (10  $\mu$ g/disc, Liofilchem, Italy) and Nystatin (100 IU/disc, Liofilchem, Italy). The discs impregnated with the antibacterial and antifungal agents were placed on the plates inoculated with the suspension of each bacteria/yeast.

Finally, all the plates were incubated at 30 °C for 24 h for bacterial tests and 48 h for tests with yeasts. Afterwards, the plates were examined for the presence of zones of inhibition of microbial growth and the diameters of the clear zones (halos) were measured in mm and recorded as the mean  $\pm$  standard deviation (SD). Each assay in this experiment was replicated three times.

## **2.4. Statistical analysis**

Data were examined by univariate analysis and the differences among the means were compared by Tukey's multiple comparison test, at significance level of  $p < 0.05$ , using the SPSS software for Windows Version 28. All data obtained in the study were as mean value  $\pm$  standard deviation (S.D.).

### **3. Results and discussions**

### **3.1. Physico-chemical analysis**

#### **3.1.1. Physico-chemical properties**

Different physico-chemical properties of the four different fruit vinegar samples (Fig enxario, Fig, Sweet potato and Grape) were analyzed throughout this work and are shown in table 3.1. As depicted in table 3.1, pH levels of the studied vinegars ranged from 2.83 to 3.49, while the Fig vinegar had the highest level. Also, as can be seen, no significant difference was observed between Fig enxario vinegar and Fig vinegar in term of pH level. According to Bakir *et al.* (2017), the pH of various fruit vinegars ranges from 2.8 to 3.9. Ozturk *et al.* (2015) also investigated the pH of several fruit vinegars and observed pH values ranging from 2.63 to 3.9.

Total acidity levels of the vinegar samples used in this study were measured and are presented in table 3.1. As a result of this study, titratable acidity of the vinegar samples was determined between 4.84% and 6.75% acetic acid and significant differences were found among the vinegar samples in terms of total acidity level ( $p < 0.05$ ). In the European Union (OJEU, 2015), vinegars should have at least 4% acetic acid (w/v). The current study's findings verified that the acidity of vinegar samples is within acceptable limits (table 3.1).

Moreover, brix values of the vinegar samples were analyzed (table 3.1). In this study, brix values of the samples varied from 4.80 to 11.20. The lowest value of total soluble solids ( $^{\circ}$ Brix) was found in Grape vinegar (4.80) while the highest value was in Fig vinegar (11.20) ( $p < 0.05$ ). Budak (2015) and Ozturk *et al.* (2015) reported that this characteristic of the fruit vinegars studied varied from 1.02 to 20.80.

#### **3.1.2. Bioactive properties**

Bioactive properties, namely total phenolic content (TPC), total flavonoid contents (TFC) and DPPH radical scavenging activities of four different vinegar samples (Fig enxario, Fig, Sweet potato and Grape) were determined by spectrophotometric methods (table 3.2). Total phenolic contents of the vinegars ranged from 0.35 gallic acid equivalent (GAE)/ml to 2.03 mg GAE/ml, while the highest value was found in the Fig vinegar and the lowest value was found in the Sweet potato vinegar.

**Table 3. 1** Physicochemical properties of vinegar samples from Algarve region.

<b>Vinegar samples</b>	<b>pH</b>	<b>Total acidity (%acetic acid(w/v))</b>	<b>°Brix</b>
<b>Fig enxario</b>	3.46 ± 0.00 <sup>a</sup>	4.84 ± 0.07 <sup>d</sup>	8.40 ± 0.57 <sup>b</sup>
<b>Fig</b>	3.49 ± 0.04 <sup>a</sup>	5.74 ± 0.21 <sup>c</sup>	11.20 ± 0.28 <sup>a</sup>
<b>Sweet potato</b>	2.91 ± 0.00 <sup>b</sup>	6.39 ± 0.08 <sup>b</sup>	5.25 ± 0.35 <sup>c</sup>
<b>Grape</b>	2.83 ± 0.01 <sup>c</sup>	6.75 ± 0.16 <sup>a</sup>	4.80 ± 0.28 <sup>c</sup>

The values are given as mean ± standard deviation of triplicate determinations. Different superscripts within the same column are significantly different ( $p < 0.05$ ).

The bioactive properties of vinegars may vary in a wide range depending on the type of raw material. According to table 3.2, there was significant difference in the levels of TPC among the four different vinegars of this study ( $p < 0.05$ ). Kelebek *et al.* (2017) reported that the substrate selection in the vinegar production process is an important parameter to consider the final phenolic content of fruit vinegars.

Total flavonoid contents (TFC) of the vinegar samples were obtained in the range of 93.68-357.31 mg catechin/ml (table 3.2). As presented in table 2, there is no significant difference in the TFC of two groups of vinegars, namely, vinegars of Fig enxario and Sweet potato, and vinegars of Fig and Grape. Among the vinegar samples, the Grape vinegar and Fig enxario vinegar exhibited the highest and the lowest TFC levels, respectively.

The antioxidant activity of the vinegars was determined based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. As it is shown in table 3.2, antioxidant activities of the vinegar samples varied from 0.05 mg TE/ml to 0.39 mg TE/ml, while the Grape vinegar had the highest value. Table 3.2 shows that there is no significant difference among vinegars ( $p < 0.05$ ), apart from Fig vinegar and Sweet potato vinegar.

**Table 3. 2.** Bioactive properties of vinegar samples from Algarve region.

<b>Vinegar samples</b>	<b>Total phenolic contents (mg GAE/ml)</b>	<b>Total flavonoid contents (mg catechin/ml)</b>	<b>Antioxidant activity (DPPH, mg TE/ml)</b>
<b>Fig enxario</b>	0.63 ± 0.01 <sup>a</sup>	93.68 ± 5.78 <sup>b</sup>	0.28 ± 0.01 <sup>b</sup>
<b>Fig</b>	2.03 ± 0.17 <sup>c</sup>	315.27 ± 6.74 <sup>a</sup>	0.05 ± 0.05 <sup>c</sup>
<b>Sweet potato</b>	0.35 ± 0.01 <sup>b</sup>	102.31 ± 5.78 <sup>b</sup>	0.12 ± 0.03 <sup>c</sup>
<b>Grape</b>	1.12 ± 0.23 <sup>d</sup>	357.31 ± 38.56 <sup>a</sup>	0.39 ± 0.02 <sup>a</sup>

The values are given as mean ± standard deviation of triplicate determinations. Different superscripts within the same column are significantly different ( $p < 0.05$ ).

### 3.1.3. Color parameters

The color of a food product is the first quality parameter evaluated by the consumer, so it is important to study the effect that food processing has on this characteristic (López *et al.*, 2005). In this study color properties of four different vinegars (Fig enxario, Fig, Sweet potato and Grape) (figure 2.1) were evaluated through the parameters of lightness ( $L^*$ ) ranged from 0 to 100, where 0 was considered as completely opaque and 100 as completely transparent. Furthermore, positive values of  $a^*$  were given for reddish colors and negative values were given to greenish colors. Positive values of  $b^*$  are associated given to yellowish color and negative values were given to bluish color.

Color properties of the vinegar samples are given in table 3.3. Based on the current study,  $L^*$  values of the tested vinegar samples ranged from 25.47 to 28.36, and values of  $a^*$  and  $b^*$  were found in the range of -0.49-0.48 and 2.01-3.23, respectively ( $p < 0.05$ ). Sweet potato vinegar had the highest  $L^*$  value. Results obtained from our experiment demonstrated that there is significant difference ( $p < 0.05$ ) among the vinegars, except for Fig enxario and Grape vinegar.

The lowest whiteness/darkness was found in Fig vinegar, which has the highest total phenolic content. Many studies have found a vast range of color characteristics for different types of

vinegars. According to Cruz *et al.* (2018), vinegars with higher phenolic content exhibit lower whiteness/darkness values.

**Table 3. 3.** Color parameters of vinegar samples from Algarve region.

<b>Vinegar samples</b>	<b>L*</b>	<b>a*</b>	<b>b*</b>
<b>Fig enxario</b>	26.85 ± 0.20 <sup>b</sup>	0.48 ± 0.10 <sup>a</sup>	3.17 ± 0.11 <sup>a</sup>
<b>Fig</b>	25.47 ± 0.93 <sup>c</sup>	0.29 ± 0.10 <sup>b</sup>	2.01 ± 0.05 <sup>c</sup>
<b>Sweet potato</b>	28.36 ± 0.29 <sup>a</sup>	-0.49 ± 0.05 <sup>c</sup>	2.89 ± 0.08 <sup>b</sup>
<b>Grape</b>	26.89 ± 0.13 <sup>b</sup>	0.44 ± 0.04 <sup>a</sup>	3.23 ± 0.11 <sup>a</sup>

The values are given as mean ± standard deviation of pentaplicate determinations. Different superscripts in the same column mean significantly different ( $p < 0.05$ ). Statistical analysis was applied separately for L\*, a\*, b\* values.

## 3.2. Microbial analysis

### 3.2.1. Microbial quality evaluation

Results from the enumeration of mesophilic aerobic microorganisms showed that the population of these microorganisms was under the detection limit (1 CFU/ml). Besides, the enumeration results revealed that the number of LAB and AAB, yeasts and filamentous fungi are also under detection limits (1 CFU/ml) in Fig enxario, Fig, Sweet potato and Grape vinegars (table 3.4) (figure 3.1).

In a similar study, Sengun (2013) reported that the counts of total mesophilic aerobic bacteria (TMAB), yeasts, LAB and AAB of eight commercial fruit vinegars including Fig vinegar, Grape vinegar, Apple vinegar, Plum vinegar and Blackberry vinegar ranged from 2.26 to 7.29 log CFU/ml, <1.00 to 6.49 log CFU/ml, 0.81 to 8.20 log CFU/ml and 2.68 to 8.23 log CFU/ml, respectively, while the samples were negative for mold, *S. aureus*, *Listeria monocytogenes*,

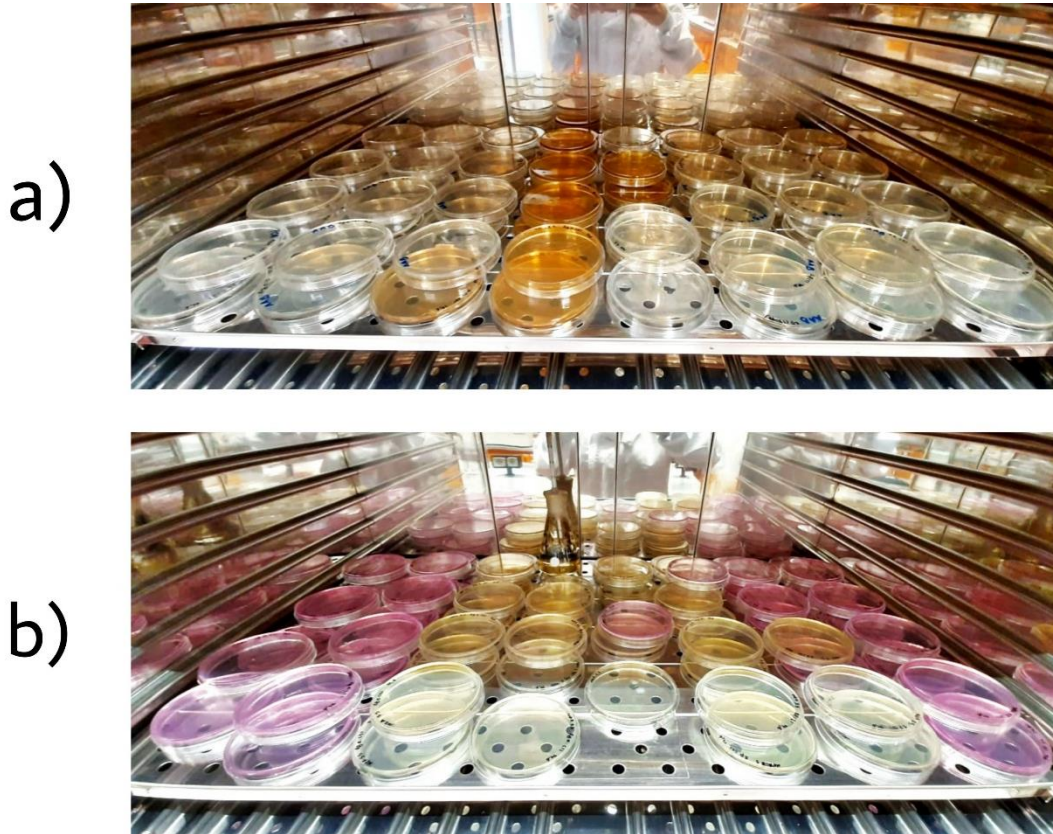
**Table 3. 4.** Enumeration of microorganisms in the vinegars (Fig enxario, Fig, Sweet potato and Grape vinegar) (CFU/ml)

Microorganisms	Fig enxario	Fig	Sweet potato	Grape
N° Samples	3	3	3	3
Total Mesophilic Aerobic Microorganisms	< 1.0	< 1.0	< 1.0	< 1.0
Acetic Acid Bacteria (AAB)	< 1.0	< 1.0	< 1.0	< 1.0
Lactic Acid Bacteria (LAB)	< 1.0	< 1.0	< 1.0	< 1.0
Filamentous Fungi	< 1.0	< 1.0	< 1.0	< 1.0
Yeasts	< 1.0	< 1.0	< 1.0	< 1.0

*Salmonella spp.*, *E. coli* and *B. cereus*. AAB belonging to the genus of *Acetobacter* are the basic bacteria that cause vinegar to be developed as ethanol is oxidized to acetic acid (Ilabaca *et al.*, 2008). As a result, numerous investigations have been carried out in order to assess the diversity of AAB in vinegar processing (Gullo *et al.*, 2006; Ilabaca *et al.*, 2008; Nanda *et al.*, 2001).

According to a recent study by Sengun *et al.* (2019) on microbial quality assessment, the total amount of LAB was enumerated in the range of between 1.60 and 5.39 log CFU/ml for Apple, Fig, Plum, Grape and Blackberry vinegars. The numbers of AAB and yeast-mold were found in the range of 3.34–6.32 and 2.53–3.97 log CFU/ml, respectively for Apple, Fig, Plum and Grape vinegar. On the other hand, LAB, AAB and yeast-mold counts of Apricot, Mandarin, Persimmon, Pomegranate and Rosehip vinegars were found under detection limits (Sengun *et al.*, 2019). In another work, the counts of AAB, LAB and yeast-mold of 20 traditional fruit vinegars (including Grape, Apple, Artichoke, Pomegranate, Apple-lemon and Hawthorn vinegars) were found in the range of  $<10-7.2 \times 10^6$ ,  $<10-1.1 \times 10^9$  and  $<10-3.9 \times 10^6$  CFU/ml, respectively (Ozturk *et al.*, 2015). It has been stated that the microbiological status of vinegar could change depending on both internal (media composition) and external (temperature and humidity) factors (Sengun *et al.*, 2019). Ethanol and acid, produced mostly by yeast (*Saccharomyces cerevisiae*) and LAB in the early stages of natural fermentation, respectively, prevent the growth of unwanted microbes and lengthen the shelf-life of vinegar (Sengun *et al.*, 2019). Following that, in the second stage of vinegar fermentation, AAB produces acetic acid, which inhibits acid-intolerant microorganisms

(Sengun *et al.*, 2019). However, due to the difficulty of control, vinegar produced by natural fermentation poses a significant danger of spoiling (Solieri and Giudici, 2009).



**Figure 3.1.** Plate Agar samples incubation: a) AAB, filamentous fungi and yeasts samples at 25 °C and b) Mesophilic Aerobic Microorganisms and LAB samples at 30 °C

Results obtained from microbial quality evaluation studies revealed very favorable microbial quality in the tested vinegar samples compared to different other studies (table 3.4). It is known that the vinegars' physico-chemical and microbial characteristics varies during different stages of fermentation processes. Detection of very low number of various microbial populations tested in this study can be due to performing the experiment on the final vinegar products, since the physicochemical parameters produced in fermentation processes affect the final quality of vinegars (Li *et al.*, 2015). Among different physico-chemical factors, acidity and pH are among the important factors that inhibit the growth of microorganisms (Gosh *et al.*, 2012). The antibacterial

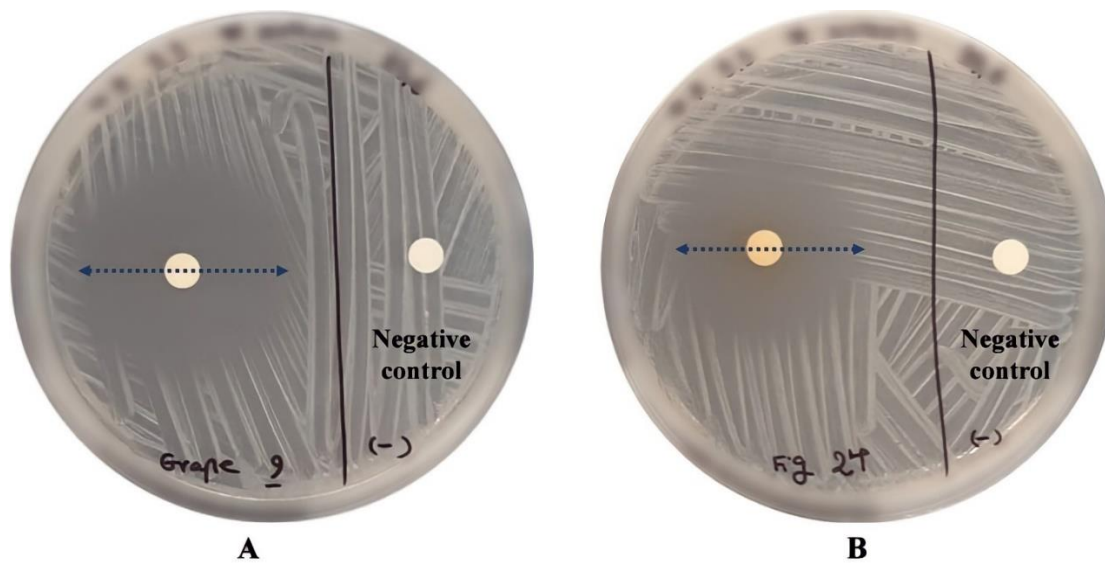
activity of acetic acid is known to be stronger at lower pH values and therefore, vinegars with higher acidities present more antibacterial properties (Li *et al.*, 2015) and by contrast, vinegars with higher pH values, have less antibacterial activity and are more capable to retain most sensory odors and volatile compounds (Jo *et al.* 2013). Moreover, the different types of molds involved in the initial stage of vinegar production, play an important role on the quality of final vinegar since they are able to produce a wide range of metabolites with antibiotic properties (Li *et al.*, 2015).

### 3.2.2. Antibacterial activity of the vinegar samples

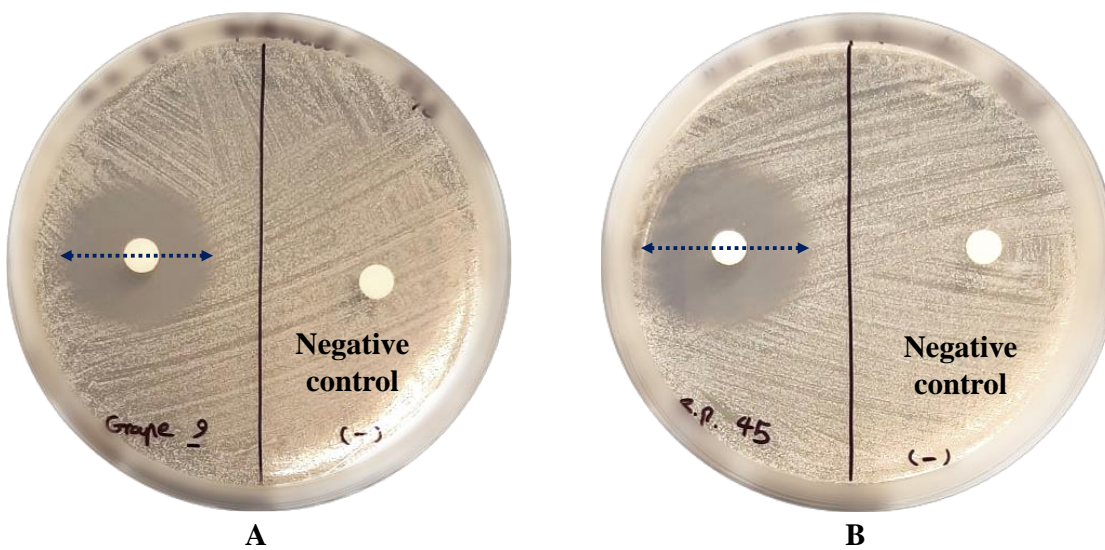
Many factors, such as pH, total acidity, bacterial strains, and environment, are capable to affect the antimicrobial activity of organic acids and acidified products and limit microbial growth, depending on the types of microorganisms existing in the product and the kind and amount of acid, particularly its buffering capacity (Bjornsdottir *et al.*, 2006).

In this study, the antimicrobial activity of four different vinegar samples (Fig enxario, Fig, Sweet potato and Grape) was investigated against eight bacteria (Gram-positive and Gram-negative bacteria) and seven yeast strains, by using disk diffusion assay.

Table 3.5. shows antibacterial activity of vinegar samples against bacteria namely *S. aureus* ATCC 25923, *L. innocua* ATCC 33090, *C. sakazakii* ATCC 29544, *S. Typhimurium* ATCC 14028, *E. coli* ATCC 25922, *S. epidermidis* ATCC 12228, *B. cereus* ATCC 10876 and *P. aeruginosa* ATCC 27853. As given in table 3.5, in general, when in contact with the vinegars studied, the Gram-positive bacteria showed growth inhibition halos higher than those measured in the Gram-negative bacteria, with the exception of *P. aeruginosa* ATCC 27853. The growth of *S. aureus* bacteria was most strongly inhibited by Grape (32.50 mm  $\pm$  0.58) and Fig vinegars (30.33 mm  $\pm$  0.88) (figure 3.2), and the values of the halos were not significantly different ( $P > 0.05$ ). The vinegar that mostly inhibited *L. innocua* was the Grape vinegar (30.33 mm  $\pm$  0.33) ( $P < 0.05$ ), followed by Sweet potato (28.17 mm  $\pm$  0.40) and Fig vinegars (28.67 mm  $\pm$  0.33) with similar inhibitions ( $P > 0.05$ ) and finally the Fig enxario vinegar (25.83 mm  $\pm$  0.17). Regarding *S. epidermidis*, the highest levels of inhibition were observed in Grape and Sweet potato vinegars which resulted in halos having similar diameters ( $P > 0.05$ ) of 25.83 mm  $\pm$  0.83 and 23.67 mm  $\pm$  1.00, respectively (figure 3.3). Grape and Fig vinegars caused the highest level of inhibition on the Gram-positive bacteria



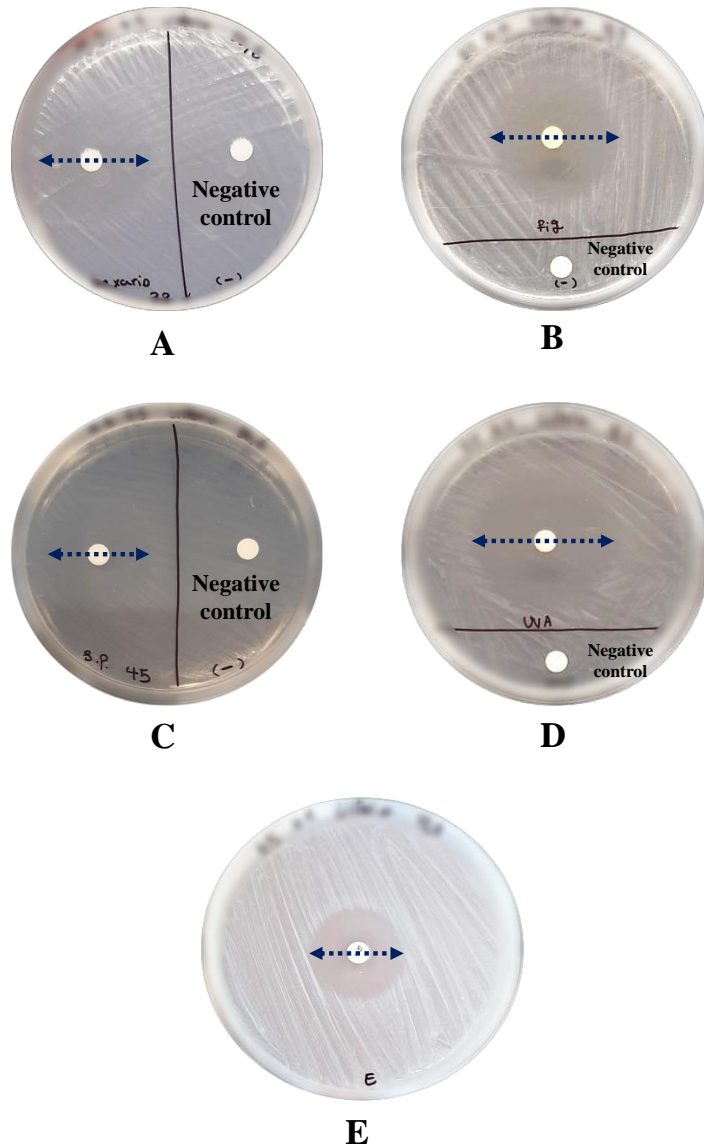
**Figure 3.2.** Antibacterial activity of A) Grape vinegar and B) Fig vinegar against *S. Aureus* ATCC 25923



**Figure 3.3.** Antibacterial activity of A) Grape vinegar and B) Sweet potato vinegar against *S. epidermidis* ATCC 12228

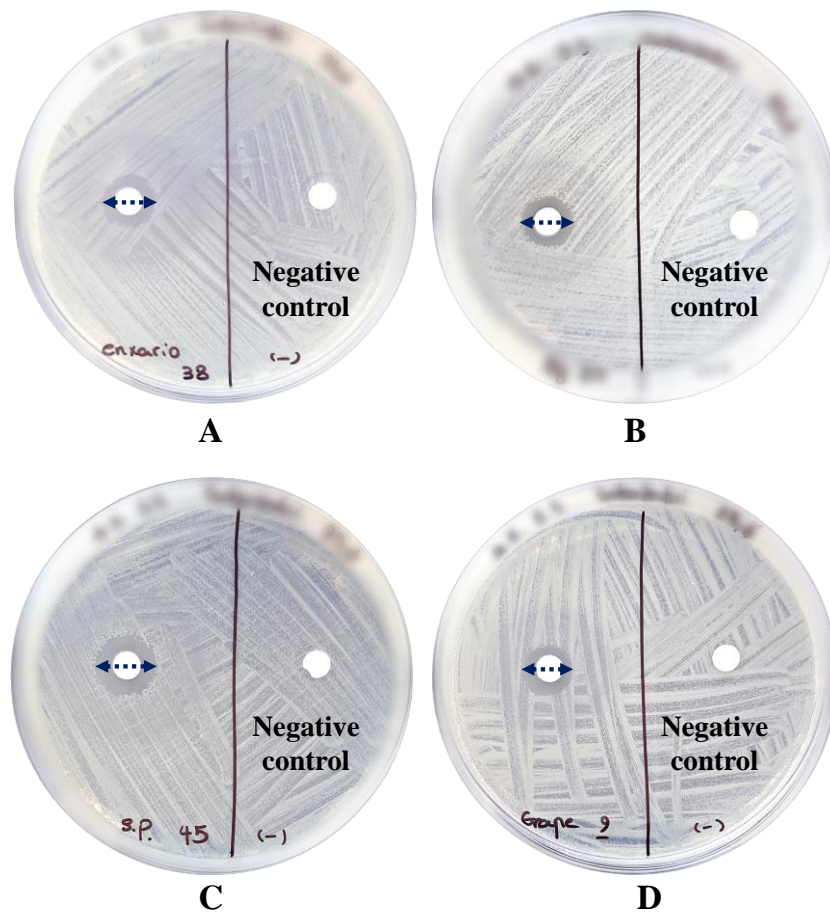
*B. cereus*, giving origin to halos of  $25.22 \text{ mm} \pm 1.19$  and  $22.83 \text{ mm} \pm 1.09$ , respectively, with no significant differences between these values ( $P > 0.05$ ). The antibiotics used as positive controls,

Ampicillin, Penicillin and Chloramphenicol, induced higher inhibitions on the Gram-positive bacteria than the vinegars tested (table 3.6). However, some of the vinegars caused higher inhibitions than the antibiotic Erythromycin (figure 3.4), such as the Grape vinegar, over all the Gram-positive bacteria tested.



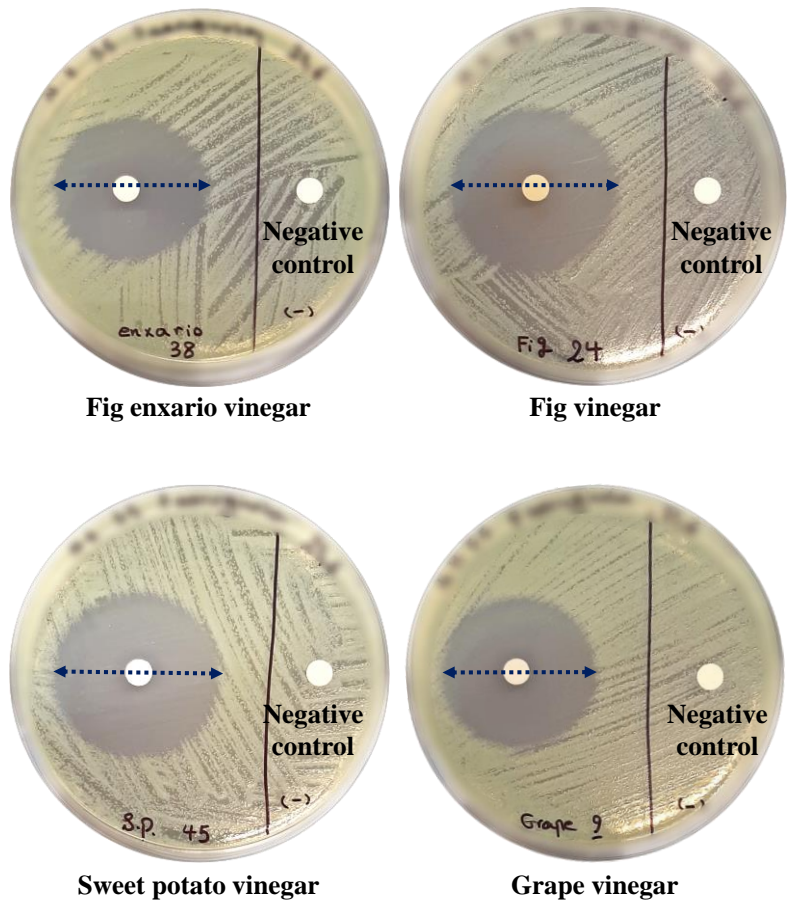
**Figure 3.4.** Comparison between the growth inhibition of the vinegar samples studied in this work: A) Fig enxario vinegar, B) Fig vinegar, C) Sweet potato vinegar, D) Grape vinegar, and E) Erythromycin against *L. innocua* ATCC 33090

Of all the Gram-negative bacteria tested, *S. Typhimurium* was the most tolerant to the vinegars tested, measuring the smallest growth inhibition zones, followed by *C. sakazakii* and *E. coli*. Sweet potato vinegar was the most efficient vinegar against *S. Typhimurium* ( $16.67 \text{ mm} \pm 0.44$ ) ( $P < 0.05$ ) followed by Grape, Fig and Fig enxario at similar levels ( $P > 0.05$ ) which caused growth inhibition halos of  $10.83 \text{ mm} \pm 0.17$ ,  $9.67 \text{ mm} \pm 0.33$  and  $10.50 \text{ mm} \pm 0.41$ , respectively. Sweet potato vinegar was also the most efficient vinegar in inhibiting the growth of *C. sakazakii* and *E. coli*. In the case of *C. sakazakii*, Sweet potato vinegar induced a halo of  $24.67 \text{ mm} \pm 1.45$  that was significantly higher ( $P < 0.05$ ) than the inhibition caused by Grape ( $11.00 \text{ mm} \pm 0.58$ ), Fig ( $11.33 \text{ mm} \pm 0.33$ ) and Fig enxario ( $12.33 \text{ mm} \pm 0.42$ ) vinegars (figure 3.5).

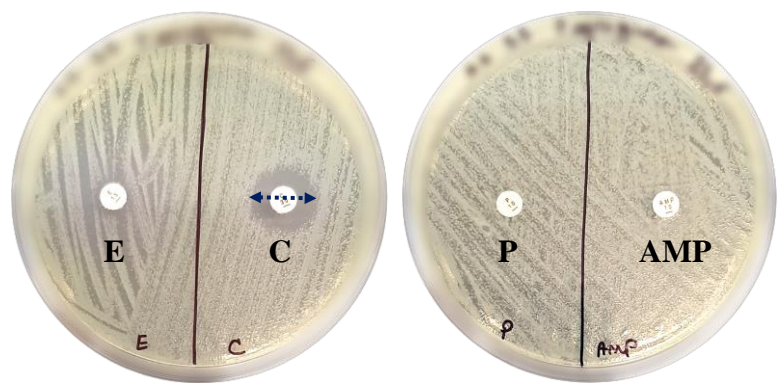


**Figure 3.5.** Antibacterial activity of vinegar samples studied in this work: A) Fig enxario vinegar, B) Fig vinegar, C) Sweet potato vinegar and D) Grape vinegar against *C. sakazakii* ATCC 29544

In regard to *E. coli*, a similar pattern was found, with Sweet potato vinegar being the most inhibitory ( $26.33 \text{ mm} \pm 0.33$ ) and Fig enxario the least efficient ( $10.67 \text{ mm} \pm 0.17$ ). *P. aeruginosa* was the most sensitive bacteria tested of all the Gram-negative and Gram-positive, showing the larger growth inhibition halos, not significantly different ( $P < 0.05$ ) in relation to the 4 vinegars tested ( $30.38 \text{ mm} \pm 0.55$  -  $35.50 \text{ mm} \pm 0.29$ ). The antibiotics used as positive controls, Ampicillin and Chloramphenicol, induced higher inhibitions on the Gram-negative *C. sakazakii*, *S. Typhimurium* and *E. coli*, than the vinegars tested. However Sweet potato and Grape vinegars induced highest inhibitions on the growth of *C. sakazakii*, *S. Typhimurium* and *E. coli* than the antibiotic Erythromycin to which they are resistant. In addition, the inhibitions caused by Penicillin on those Gram-negative bacteria were lower than those of some of the vinegars. Regarding *P. aeruginosa*, which is resistant to Ampicillin and Penicillin, all the tested vinegars caused greater inhibitions than the antibiotics (Chloramphenicol and Erythromycin) used as positive controls (figure 3.6).



**A**



**E: Erythromycin**  
**C: Chloramphenicol**

**P: Penicillin**  
**AMP: Ampicillin**

**B**

**Figure 3.6.** Comparison between the growth inhibition by A) vinegar samples and B) antibiotics against *P. aeruginosa* ATCC 27853

The antimicrobial activity of vinegars has also been reported by several investigators using the agar diffusion method. For example, Kadiroğlu (2018) used this method by applying different commercial vinegar samples including Balsamic, Rice, Apple, Red and White wine, Red and White grapes and Pomegranate vinegars against *S. aureus*, *E. coli* and *P. aeruginosa* and observed the lowest antibacterial activity by Apple vinegar against all the mentioned bacteria. Moreover, their study revealed the Balsamic vinegar with highest antibacterial activity against *S. aureus* and White wine and Grape vinegars with the highest antibacterial activity against *E. coli* and *P. aeruginosa*, respectively. In another study, Chan *et al.* (2012) showed that Wood vinegar has high antibacterial activity against the strains of Gram-positive *B. cereus*, *Micrococcus luteus* and *S. aureus*, and Gram-negative *E. coli*, *S. Typhimurium* ATCC 14028, and *P. aeruginosa*. Tumane *et al.* (2018) also reported that Apple cider vinegar has high antibacterial activity against different strains of Gram-positive and Gram-negative bacteria.

There are studies suggesting that the antimicrobial activity of the vinegars can be increased by lowering the pH of the media due to susceptibility of most of the microorganisms to the antimicrobial effects of the vinegars in the presence of organic acids (Osuala *et al.*, 2021). Among organic acids, acetic acid presents strong antimicrobial activities (Hassan *et al.* 2015). Hassan *et al.* (2015) reported that organic acids resulted in a decrease in pH, which can influence the growth by acidifying the cells, which will consume a great amount of energy to maintain the intracellular pH homeostasis. Acetic acid in vinegar is a particularly effective antimicrobial, because at a relatively high pH (pH=4.7) it exists primarily in its undissociated form and can enter the cells (Hirshfield *et al.*, 2003). Budak *et al.* (2014) mentioned that the antimicrobial activity of organic acid can be influenced by the target bacterial strains, temperature, pH, acid concentration and ionic strength.

**Table 3. 5.** Antibacterial activity of the vinegar samples from Algarve region against some bacterial pathogens (inhibition zone mm).

Vinegar samples	<i>S. aureus</i> ATCC 25923	<i>L. innocua</i> ATCC 33090	<i>C. sakazakii</i> ATCC 29544	<i>S. Typhimurium</i> ATCC 14028	<i>E. coli</i> ATCC 25922	<i>S. epidermidis</i> ATCC 12228	<i>B. cereus</i> ATCC 10876	<i>P. aeruginosa</i> ATCC 27853
<b>Fig enxario</b>	25.08 ± 0.68 <sup>b</sup>	25.83 ± 0.17 <sup>c</sup>	12.33 ± 0.42 <sup>b</sup>	10.50 ± 0.41 <sup>b</sup>	10.67 ± 0.17 <sup>d</sup>	19.50 ± 0.34 <sup>b</sup>	16.67 ± 1.36 <sup>b</sup>	30.38 ± 0.55 <sup>a</sup>
<b>Fig</b>	30.33 ± 0.88 <sup>a</sup>	28.67 ± 0.33 <sup>b</sup>	11.33 ± 0.33 <sup>b</sup>	9.67 ± 0.33 <sup>b</sup>	12.00 ± 0.00 <sup>c</sup>	22.83 ± 0.73 <sup>ab</sup>	22.83 ± 1.09 <sup>a</sup>	32.17 ± 0.83 <sup>a</sup>
<b>Sweet potato</b>	26.67 ± 0.33 <sup>b</sup>	28.17 ± 0.40 <sup>b</sup>	24.67 ± 1.45 <sup>a</sup>	16.67 ± 0.44 <sup>a</sup>	26.33 ± 0.33 <sup>a</sup>	23.67 ± 1.00 <sup>a</sup>	21.50 ± 1.26 <sup>ab</sup>	32.67 ± 1.30 <sup>a</sup>
<b>Grape</b>	32.50 ± 0.58 <sup>a</sup>	30.33 ± 0.33 <sup>a</sup>	11.00 ± 0.58 <sup>b</sup>	10.83 ± 0.17 <sup>b</sup>	15.83 ± 0.44 <sup>b</sup>	25.83 ± 0.83 <sup>a</sup>	25.22 ± 1.19 <sup>a</sup>	35.50 ± 0.29 <sup>a</sup>

Standard deviation of means is shown as ± SD. Values in the same column with different superscripts are significantly different ( $p < 0.05$ )

**Table 3. 6.** Antibacterial activity of different antibiotics against some bacterial pathogens (inhibition zone. mm)

Antibiotics	<i>S. aureus</i> ATCC 25923	<i>L. innocua</i> ATCC 33090	<i>C. sakazakii</i> ATCC 29544	<i>S. Typhimurium</i> ATCC 14028	<i>E. coli</i> ATCC 25922	<i>S. epidermidis</i> ATCC 12228	<i>B. cereus</i> ATCC 10876	<i>P. aeruginosa</i> ATCC 27853
<b>Ampicillin</b>	36.15 ± 0.77 <sup>b</sup>	35.70 ± 1.36 <sup>a</sup>	28.10 ± 0.57 <sup>b</sup>	30.10 ± 0.52 <sup>b</sup>	24.15 ± 0.52 <sup>b</sup>	28.80 ± 0.96	28.83 ± 3.15 <sup>ab</sup>	0.00 ± 0.00 <sup>c</sup>
<b>Penicillin</b>	42.20 ± 0.57 <sup>a</sup>	36.55 ± 1.63 <sup>a</sup>	8.38 ± 0.53 <sup>c</sup>	19.10 ± 0.73 <sup>c</sup>	7.88 ± 0.31 <sup>c</sup>	23.22 ± 0.60	22.41 ± 2.38 <sup>b</sup>	0.00 ± 0.00 <sup>c</sup>
<b>Chloramphenicol</b>	31.10 ± 0.67 <sup>c</sup>	35.25 ± 0.63 <sup>a</sup>	30.15 ± 0.22 <sup>a</sup>	32.95 ± 0.63 <sup>a</sup>	32.45 ± 0.61 <sup>a</sup>	36.39 ± 0.93	31.82 ± 1.07 <sup>a</sup>	12.36 ± 0.52 <sup>b</sup>
<b>Erythromycin</b>	19.70 ± 0.40 <sup>d</sup>	25.00 ± 0.75 <sup>b</sup>	0.00 ± 0.00 <sup>d</sup>	0.00 ± 0.00 <sup>d</sup>	0.00 ± 0.00 <sup>d</sup>	23.83 ± 0.86	23.27 ± 0.43 <sup>b</sup>	19.50 ± 1.73 <sup>a</sup>

Standard deviation of means is shown as ± SD. Values in the same column with different superscripts are significantly different ( $p < 0.05$ )

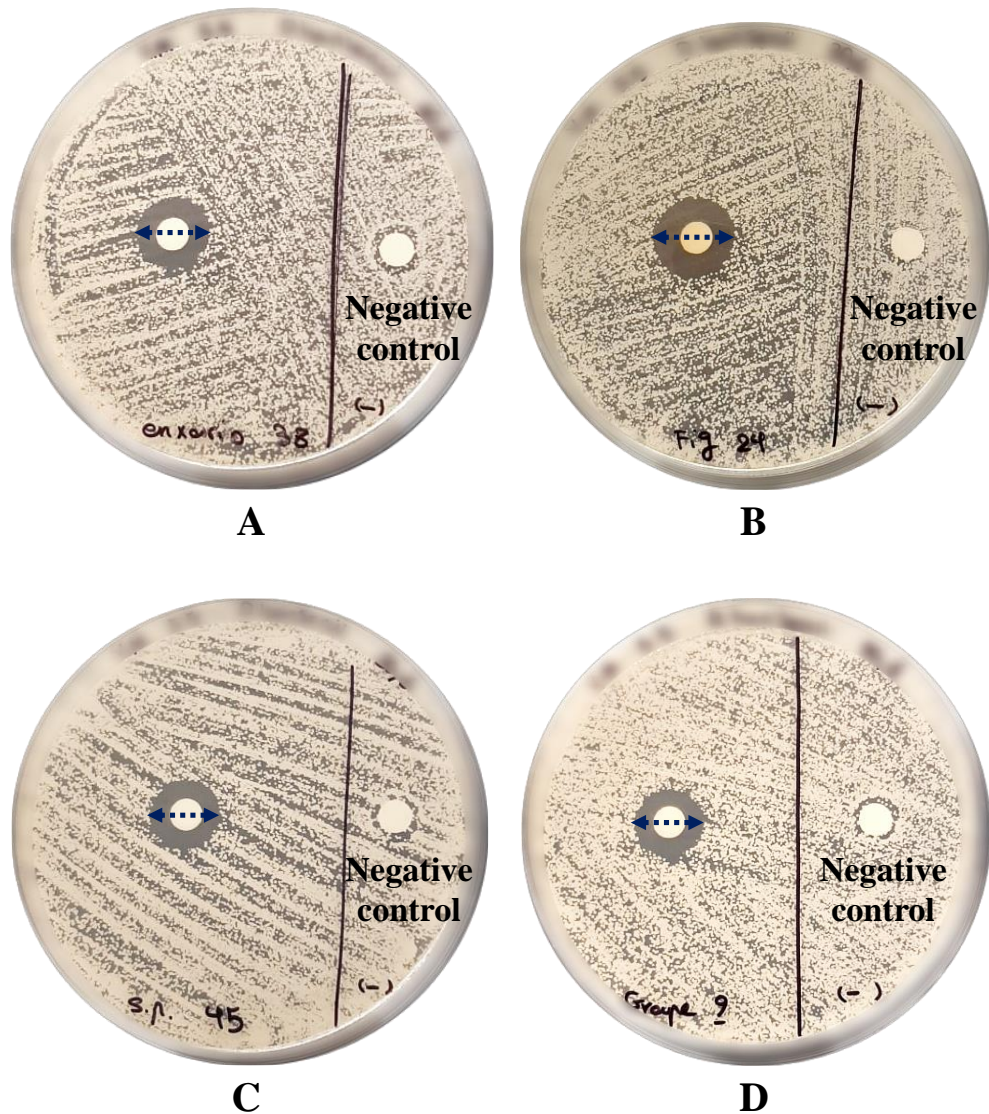
### 3.2.3. Antifungal activity of the vinegar samples

Vinegar samples (Fig enxario, Fig, Sweet potato and Grape) were also examined against some yeasts including *Candida albicans* ATCC 10231, *Candida albicans* ATCC 90028, *C. neoformans* YPO 186, *S. cerevisiae* PYCC 3507, *P. membranifaciens* PYCC 2489, *Z. bailii* PYCC 4806 and *D. hansenii* PYCC 2968. The obtained results for antifungal properties are summarized in table 3.6.

The data on table 3.7 showed that the vinegars caused levels of inhibition of yeast growth much lower than those caused in the bacteria studied (table 3.5). The yeasts tested were more tolerant to vinegars than bacteria. For example, the species *C. albicans* ATCC 10231, *S. cerevisiae*, *Z. bailii* were not inhibited by any of the vinegars, being highly tolerant to this kind of product. The species *P. membranaifaciens* was not inhibited by Fig enxario and Sweet potato vinegars. The highest inhibition halo ( $7.17 \text{ mm} \pm 0.17$ ) was measured in this yeast in the presence of Grape vinegar. With regard to *C. neoformans*, Sweet potato vinegar was the most inhibitory ( $P < 0.05$ ) ( $12.33 \text{ mm} \pm 0.33$ ). The yeast *D. hansenii* was the most sensitive to the studied vinegars, with the Sweet potato and Grape vinegars inducing the greatest inhibitory actions ( $14.33 \text{ mm} \pm 0.33$  and  $13.39 \text{ mm} \pm 0.47$ , respectively) (figure 3.7).

In the present study, fungicidal activity against *C. albicans* ATCC 10231, *S. cerevisiae* and *Z. bailii* was not found in any vinegar sample. On the contrary, growth of *D. hansenii* was considerably affected by all vinegar samples, while the highest level of growth-inhibitory effect was observed for Sweet potato vinegar ( $14.33 \text{ mm} \pm 0.33$ ) (table 3.6).

Furthermore, antifungal properties of three antimycotics including Fluconazole 25, Amphotericin B10 and Nystatin 100 were measured and are given in table 3.8. As depicted in table 3.8, *C. albicans* ATCC 10231, *P. membranifaciens* and *Z. bailii* were resistant to Fluconazole 25. However, the mentioned antimycotic had the highest inhibitory effect towards *D. hansenii*, with inhibition zone of  $31.92 \text{ mm} \pm 2.19$ . Nystatin 100 was the only antimycotic that affected the growth of all tested yeasts (table 3.8).



**Figure 3.7.** Antifungal activity of vinegar samples A) Fig enxario vinegar, B) Fig vinegar, C) Sweet potato vinegar and D) Grape vinegar against *D. hansenii* PYCC 2968

Antimicrobial activity of vinegars from different origin is reported by various researchers. In a study conducted by Kelebek *et al.* (2017), a Grape vinegar had a considerable growth-inhibitory effect against *S. aureus* ATCC 29213, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. Moreover, phenolic compounds in fruits and vegetables provide significant antimicrobial properties (Cowan, 1999; Fernández-Agulló *et al.*, 2013). Gallic acid is mentioned to have high antimicrobial activity as well as its antioxidant activity (Sun *et al.*, 2014). Furthermore, naturally

occurring phenolic compounds in grapes, in addition to their high antioxidant capacities, are responsible for the high antibacterial potential of Grape vinegars (Ramos *et al.*, 2014; Kelebek *et al.*, 2017).

**Table 3. 7.** Antifungal properties of the vinegar samples from Algarve region against some yeasts (inhibition zone mm).

Vinegar samples	<i>C. albicans</i> ATCC 10231	<i>C. albicans</i> ATCC 90028	<i>C. neoformans</i> YPO 186	<i>S. cerevisiae</i> PYCC 3507	<i>P. membranifaciens</i> PYCC 2489	<i>Z. bailii</i> PYCC 4806	<i>D. hansenii</i> PYCC 2968
<b>Fig enxario</b>	0.00 ± 0.00 <sup>a</sup>	8.87 ± 0.13 <sup>b</sup>	9.83 ± 0.17 <sup>b</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	11.67 ± 0.33 <sup>b</sup>
<b>Fig</b>	0.00 ± 0.00 <sup>a</sup>	8.50 ± 0.26 <sup>b</sup>	8.50 ± 0.29 <sup>c</sup>	0.00 ± 0.00 <sup>a</sup>	6.50 ± 0.29 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	12.00 ± 0.26 <sup>b</sup>
<b>Sweet potato</b>	0.00 ± 0.00 <sup>a</sup>	9.92 ± 0.08 <sup>a</sup>	12.33 ± 0.33 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	14.33 ± 0.33 <sup>a</sup>
<b>Grape</b>	0.00 ± 0.00 <sup>a</sup>	9.04 ± 0.19 <sup>ab</sup>	9.67 ± 0.17 <sup>b</sup>	0.00 ± 0.00 <sup>a</sup>	7.17 ± 0.17 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	13.92 ± 0.47 <sup>a</sup>

Standard deviation of means is shown as ± SD. Values in the same column with different superscripts are significantly different ( $p < 0.05$ )

**Table 3. 8.** Antifungal properties of antimycotics against some yeasts (inhibition zone mm)

<b>Antimycotics</b>	<i>C. albicans</i> ATCC 10231	<i>C. albicans</i> ATCC 90028	<i>C. neoformans</i> YPO 186	<i>S. cerevisiae</i> PYCC 3507	<i>P. membranifaciens</i> PYCC 2489	<i>Z. bailii</i> PYCC 4806	<i>D. hansenii</i> PYCC 2968
<b>Fuconazole 25</b>	0.00 ± 0.00 <sup>c</sup>	30.45 ± 1.74 <sup>a</sup>	15.53 ± 1.09 <sup>b</sup>	12.80 ± 1.24 <sup>b</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	31.92 ± 2.19 <sup>a</sup>
<b>Amphotericin B 10</b>	23.00 ± 0.42 <sup>b</sup>	16.70 ± 0.21 <sup>b</sup>	13.63 ± 0.32 <sup>b</sup>	10.40 ± 0.81 <sup>b</sup>	8.13 ± 0.30 <sup>b</sup>	10.44 ± 0.37 <sup>b</sup>	0.00 ± 0.00 <sup>c</sup>
<b>Nystatin 100</b>	29.65 ± 0.61 <sup>a</sup>	17.70 ± 0.47 <sup>b</sup>	26.19 ± 0.38 <sup>a</sup>	23.56 ± 0.80 <sup>a</sup>	24.06 ± 1.41 <sup>a</sup>	21.56 ± 0.86 <sup>a</sup>	8.67 ± 0.51 <sup>b</sup>

Standard deviation of means is shown as ± SD. Values in the same column with different superscripts are significantly different ( $p < 0.05$ )

## **4. Conclusion**

## Conclusions

Results of the present study have indicated that variety in initial raw material in vinegar production results in different characteristics, in terms of total acidity, pH, °Brix, color parameters (L\*, a\* and b\*), total phenolic content, total flavonoid content, antioxidant and antimicrobial activities among the vinegars.

Fig enxario vinegar and Grape vinegar exhibited the highest content of acetic acid and antioxidant activity, respectively. The lowest transparency was observed for Fig vinegar, which also exhibited the highest value for phenolic content and °brix.

The study showed that fruit vinegars have quite high level of bioactive compounds, which made them capable to exhibit a significant antimicrobial activity, namely against bacteria. In general, Gram-positive bacteria exhibited more sensitivity than Gram-negative ones. Among the four tested vinegars, the Grape vinegar exhibited the most growth-inhibitory effects against *P. aeruginosa* ATCC 27853. Also, the yeasts tested revealed more tolerance to the vinegars than the bacteria. Generally, the vinegars studied exhibited an excellent microbiology quality regarding all the microbiological parameters studied. This work proved that vinegars have important components which are beneficial worth to investigate.

## **5. Future perspectives**

## **Future perspectives**

This work has raised several points that merit further studies. Given the fact that limited research is available on the characterization of the different fruit vinegars, several perspectives of studies can be carried out. Some examples of future research include:

- This study focused on the characteristics of the final product (the commercialized vinegar); it would be interesting to continue this work with the aim to characterize the fermentation processes of the vinegars.
- Characterization of the composition of the vinegars in terms of organic acids, organic compounds, alcohol contents, volatile compounds, minerals and sugars.
- Evaluation of the antimicrobial activity of the vinegar samples that were examined in this study on other bacterial and fungal strains in order to gain a more extensive understanding of the antimicrobial properties of the commercialized vinegars.
- A comprehensive study on Fig enxario vinegar and the compounds present in it, could be an attractive approach, as Fig enxario is a non-studied fruit

## **6. Bibliographic references**

## References

- Akbas, M. and T. Cabaroglu. (2010). A research on the determination of compositions of grape vinegars produced in Turkey and their conformity to food legislation.
- Alam, M., Rana, Z. and Islam, S. (2016). Comparison of the Proximate Composition, Total Carotenoids and Total Polyphenol Content of Nine Orange-Fleshed Sweet Potato Varieties Grown in Bangladesh. *Foods*, 5(4), p.64.
- Alam, M.K. (2021). A comprehensive review of sweet potato (*Ipomoea batatas* [L.] Lam): Revisiting the associated health benefits. *Trends in Food Science & Technology*, 115, pp.512–529.
- Alam, M.K., Sams, S., Rana, Z.H., Akhtaruzzaman, M. and Islam, S.N. (2020). Minerals, vitamin C, and effect of thermal processing on carotenoids composition in nine varieties orange-fleshed sweet potato (*Ipomoea batatas* L.). *Journal of Food Composition and Analysis*, 92, p.103582.
- Allegra, A., Gallotta, A., Carimi, F., Mercati, F., Inglese, P. and Martinelli, F. (2018). Metabolic Profiling and Post-harvest Behavior of “Dottato” Fig (*Ficus carica* L.) Fruit Covered With an Edible Coating From *O. ficus-indica*. *Frontiers in Plant Science*, 9.
- Anonymous, (2016). Vinegar-product made from liquids of agricultural origin-definition, requirements, marking, Turkish Standards Institute, Ankara, (Vol. TS 1880 EN 13188/D1: 2016).
- Arai, H., Sakurai, K. and Ishii, M. (2016). Metabolic Features of *Acetobacter aceti*. *Acetic Acid Bacteria*, [online] pp.255–271. Available at: [https://link.springer.com/chapter/10.1007%2F978-4-431-55933-7\\_12](https://link.springer.com/chapter/10.1007%2F978-4-431-55933-7_12) [Accessed 20 Nov. 2019].
- Atinkut, H.B., Yan, T., Arega, Y. and Raza, M.H. (2020). Farmers’ willingness-to-pay for eco-friendly agricultural waste management in Ethiopia: A contingent valuation. *Journal of Cleaner Production*, 261, pp.1–18.
- Bakir, S., Devecioglu, D., Kayacan, S., Toydemir, G., Karbancioglu-Guler, F. and Capanoglu, E. (2017). Investigating the antioxidant and antimicrobial activities of different vinegars. *European Food Research and Technology*, 243(12), pp.2083–2094.

- Beijerinck, M. W., (1898). Ueber die arten der essigbakterien. *Zentralbl Bakteriol Parasitenkd Infektionskr Hyg Abt II* 2(4):209–216. (In German).
- Bekatorou, A. (2016). Alcohol: Properties and Determination. *Encyclopedia of Food and Health*, pp.88–96.
- Bekatorou, A., (2020). *Advances in vinegar production*. Boca Raton, FL: Crc Press, Taylor & Francis Group.
- Bhatt, A.H., Karanjekar, R.V., Altouqi, S., Sattler, M.L., Hossain, M.D.S. and Chen, V.P. (2017). Estimating landfill leachate BOD and COD based on rainfall, ambient temperature, and waste composition: Exploration of a MARS statistical approach. *Environmental Technology & Innovation*, 8, pp.1–16.
- Bhattarai, P., Mishra Tripathi, K., Mani Gautam, D. and Kumar Shrestha, A. (2021). Storability of sweet potato genotypes under ordinary ambient storage conditions. *Journal of Agricultural Science*, 32(2).
- Biesalski, H.-K., Dragsted, L.O., Elmadfa, I., Grossklaus, R., Müller, M., Schrenk, D., Walter, P. and Weber, P. (2009). Bioactive compounds: Definition and assessment of activity. *Nutrition*, 25(11-12), pp.1202–1205.
- Bjornsdottir, K., Breidt, F. and McFeeters, R.F. (2006). Protective Effects of Organic Acids on Survival of *Escherichia coli* O157:H7 in Acidic Environments. *Applied and Environmental Microbiology*, [online] 72(1), pp.660–664. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1352172/> [Accessed 1 May 2019].
- Boistean, A. (2021). Aspects of vinegars production and marketing in moldova. *Journal of Social Sciences*, IV(2), pp.128–138.
- Bouyahya, A, Bensaid, M, Bakri, Y & Dakka, N 2016, 'Phytochemistry and Ethnopharmacology of *Ficus carica*', *International Journal of Biochemistry Research & Review*, vol. 14, no. 1, pp. 1–12.
- Braca, A., De Tommasi, N., Di Bari, L., Pizza, C., Politi, M. and Morelli, I. (2001). Antioxidant Principles from *Bauhinia tarapotensis*. *Journal of Natural Products*, 64(7), pp.892–895.

- Bringer, S. and Bott, M. (2016). Central Carbon Metabolism and Respiration in *Gluconobacter oxydans*. *Acetic acid bacteria*, pp.235–253.
- Brown, A.J. (1886). XLIII.—On an acetic ferment which forms cellulose. *Journal of the Chemical Society, Transactions*, [online] 49(0), pp.432–439. Available at: <https://pubs.rsc.org/en/content/articlelanding/1886/ct/ct8864900432#>.
- Budak, H.N. (2015). Total Antioxidant Activity and Phenolic Contents with Advanced Analytical Techniques in the Mulberry Vinegar Formation Process. *International Journal of Fruit Science*, 2, pp.27–31.
- Budak, N.H., Aykin, E., Seydim, A.C., Greene, A.K. and Guzel-Seydim, Z.B. (2014). Functional Properties of Vinegar. *Journal of Food Science*, [online] 79(5), pp.757–764. Available at: <https://onlinelibrary.wiley.com/doi/full/10.1111/1750-3841.12434>.
- Çalışkan, O. and Aytakin Polat, A. (2011). Phytochemical and antioxidant properties of selected fig (*Ficus carica* L.) accessions from the eastern Mediterranean region of Turkey. *Scientia Horticulturae*, 128(4), pp.473–478.
- Carrascosa, A.V., MuñozR. and GonzálezR. (2011). *Molecular wine microbiology*. Amsterdam; Boston: Academic Press.
- Chen, Y., Bai, Y., Li, D., Wang, C., Xu, N., Wu, S., He, S. and Hu, Y. (2015). Correlation between ethanol resistance and characteristics of PQQ-dependent ADH in acetic acid bacteria. *European Food Research and Technology*, 242(6), pp.837–847.
- Chou, C.-H., Liu, C.-W., Yang, D.-J., Wu, Y.-H.S. and Chen, Y.-C. (2015). Amino acid, mineral, and polyphenolic profiles of black vinegar, and its lipid lowering and antioxidant effects in vivo. *Food Chemistry*, 168, pp.63–69.
- Christy, E.J.S., Suganya, K., Kiruba, J.J.G., Madhumitha, S.P., Suja\*, S.K. and Kalaivani, G.J. (2014). Extraction of Pectin from Fruit wastes– an effective method of municipal solid waste management. *International Journal of Advanced Research*, 2(2), pp.936–944.
- Cleenwerck, I. and De Vos, P. (2008). Polyphasic taxonomy of acetic acid bacteria: An overview of the currently applied methodology. *International Journal of Food Microbiology*, 125(1), pp.2–14.

- Codex Alimentarius Commission. 1987. Draft European Regional Standard for Vinegar. Geneva, Switzerland: World Health Organization.
- Colombo, F., Di Lorenzo, C., Regazzoni, L., Fumagalli, M., Sangiovanni, E., Peres de Sousa, L., Bavaresco, L., Tomasi, D., Bosso, A., Aldini, G., Restani, P. and Dell'Agli, M. (2019). Phenolic profiles and anti-inflammatory activities of sixteen table grape (*Vitis vinifera* L.) varieties. *Food & Function*, 10(4), pp.1797–1807.
- Cowan, M.M. (1999). Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews*, 12(4), pp.564–582.
- Crisosto, C.H., Bremer, V., Ferguson, L. and Crisosto, G.M. (2010). Evaluating Quality Attributes of Four Fresh Fig (*Ficus carica* L.) Cultivars Harvested at Two Maturity Stages. *HortScience*, 45(4), pp.707–710.
- Cruz, M., Correia, A.C., Gonçalves, F.J. and Jordão, A.M. (2018). Phenolic composition and total antioxidant capacity analysis of red wine vinegars commercialized in Portuguese market. *Ciência e Técnica Vitivinícola*, 33(2), pp.102–115.
- Dabija, A. and Hatnean, C.A. (2014). Study concerning the quality of apple vinegar obtained through classical method. *Journal of Agroalimentary Processes and Technologies*, 20(4), pp.304–310.
- Daglia, M. (2012). Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology*, 23(2), pp.174–181.
- Dak, E., Retta, N. and Desse, G. (2016). Comparison of Three Sweet Potato (*Ipomoea Batatas* (L.) Lam) Varieties on Nutritional and Anti-Nutritional Factors. *Global Journal of Science Frontier Research: D Agriculture and Veterinary*, 16(4).
- Dewanto, V., Wu, X., Adom, K.K. and Liu, R.H. (2002). Thermal Processing Enhances the Nutritional Value of Tomatoes by Increasing Total Antioxidant Activity. *Journal of Agricultural and Food Chemistry*, [online] 50(10), pp.3010–3014. Available at: <https://ucanr.edu/datastoreFiles/608-418.pdf>.
- Di Donna, L., Bartella, L., De Vero, L., Gullo, M., Giuffrè, A.M., Zappia, C., Capocasale, M., Poiana, M., D'Urso, S. and Caridi, A. (2020). Vinegar production from Citrus bergamia by-products and preservation of bioactive compounds. *European Food Research and Technology*, 246(10), pp.1981–1990.

- Eriksson, M., & Spångberg, J., (2017). Carbon footprint and energy use of food waste management options for fresh fruit and vegetables from supermarkets. *Waste Management*, 60, pp.786–799.
- Esparza, I., Jiménez-Moreno, N., Bimbela, F., Ancín-Azpilicueta, C. and Gandía, L.M. (2020). Fruit and vegetable waste management: Conventional and emerging approaches. *Journal of Environmental Management*, 265, p.110510.
- European Committee on Antimicrobial Susceptibility Testing, Antimicrobial susceptibility testing EUCAST disk diffusion method Version 8.0. 2020.
- FAO. (2011). Global food losses and food waste - Extent, causes and prevention. Rome: *Food and Agriculture Organization of the United Nations*.
- FAO/WHO (Food and Agriculture Organization/World Health Organization) (2002). *Guidelines for the Evaluation of Probiotics in Food*. London, Ontario.
- FAOSTAT, 2019. Food and Agriculture Organization Statistic Database. Available at <http://www.fao.org/faostat/en/#data/QC/visualize>.
- FDA (Food and Drug Administration). Vinegar, definitions—adulteration with vinegar eels, 1995. Available from: <https://www.fda.gov/ucm/groups/fdagov-public/@fdagov-afda-ice/documents/webcontent/ucm074471.pdf>. Accessed 9 Jan 2018.
- Fernández-Agulló, A., Pereira, E., Freire, M.S., Valentão, P., Andrade, P.B., González-Álvarez, J. and Pereira, J.A. (2013). Influence of solvent on the antioxidant and antimicrobial properties of walnut (*Juglans regia* L.) green husk extracts. *Industrial Crops and Products*, 42, pp.126–132.
- Flores, R. (1978). A rapid and reproducible assay for quantitative estimation of proteins using bromophenol blue. *Analytical Biochemistry*, 88(2), pp.605–611.
- Frings, 2018: Heinrich Frings GmbH & Co, Germany (www.frings.com)
- Fukami, H., Tachimoto, H., Kishi, M., Kaga, T. and Tanaka, Y. (2010). Acetic Acid Bacterial Lipids Improve Cognitive Function in Dementia Model Rats. *Journal of Agricultural and Food Chemistry*, 58(7), pp.4084–4089.
- Fushimi, T., Suruga, K., Oshima, Y., Fukiharuru, M., Tsukamoto, Y. and Goda, T. (2006). Dietary acetic acid reduces serum cholesterol and triacylglycerols in rats fed a cholesterol-rich diet. *The*

*British Journal of Nutrition*, [online] 95(5), pp.916–924. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/16611381>.

- Ghosh, S., Chakraborty, R., Chatterjee, G. and Raychaudhuri, U. (2012). Study on fermentation conditions of palm juice vinegar by response surface methodology and development of a kinetic model. *Brazilian Journal of Chemical Engineering*, 29(3), pp.461–472.
- Gilani, A.H., Mehmood, M.H., Janbaz, K.H., Khan, A. and Saeed, S.A. (2008). Ethnopharmacological studies on antispasmodic and antiplatelet activities of *Ficus carica*. *Journal of Ethnopharmacology*, 119(1), pp.1–5.
- Gomes, R.J., Borges, M. de F., Rosa, M. de F., Castro-Gómez, R.J.H. and Spinosa, W.A. (2018). Acetic Acid Bacteria in the Food Industry: Systematics, Characteristics and Applications. *Food Technology and Biotechnology*, 56(2).
- Gry, J., Black, L., Eriksen, F., Pilegaard, K., Plumb, J., Rhodes, M., Sheehan, D., Kiely, M. and Kroon, P. (2007). EuroFIR-BASIS – a combined composition and biological activity database for bioactive compounds in plant-based foods. *Trends in Food Science & Technology*, 18(8), pp.434–444.
- Guaâdaoui, A. (2014). Betalains, Polyacetylenes and Tocols as Biocompounactives: A Concise Review for Enriching the Bioactivity Concept. *International Journal of Nutrition and Food Sciences*, 3(4), p.230.
- Guaâdaoui, A., Benaïcha, S., Elmajdoub, N., Bellaoui, M. and Hamal, A. (2014). What is a Bioactive Compound? A Combined Definition for a Preliminary Consensus. *International Journal of Nutrition and Food Sciences*, 3(3), p.174.
- Gullo, M. and Giudici, P. (2008). Acetic acid bacteria in traditional balsamic vinegar: Phenotypic traits relevant for starter cultures selection. *International Journal of Food Microbiology*, 125(1), pp.46–53.
- Gullo, M., Caggia, C., De Vero, L. and Giudici, P. (2006). Characterization of acetic acid bacteria in “traditional balsamic vinegar.” *International Journal of Food Microbiology*, 106(2), pp.209–212.

- Gullo, M., De Vero, L. and Giudici, P. (2014). Succession of Selected Strains of *Acetobacter pasteurianus* and Other Acetic Acid Bacteria in Traditional Balsamic Vinegar. *Applied and Environmental Microbiology*, 75(8), pp.2585–2589.
- Hassan, R., El-Kadi, S., and Sand. M. (2015). Effect of some organic acids on some fungal growth and their toxins production. *International Journal of Advances in Biology*, 2, pp.1-11.
- Heard, G.M. and Fleet, G.H. (1988). The effects of temperature and pH on the growth of yeast species during the fermentation of grape juice. *Journal of Applied Bacteriology*, 65(1), pp.23–28.
- Hidalgo, C., Vegas, C., Mateo, E., Tesfaye, W., Cerezo, A.B., Callejón, R.M., Poblet, M., Guillamón, J.M., Mas, A. and Torija, M.J. (2010). Effect of barrel design and the inoculation of *Acetobacter pasteurianus* in wine vinegar production. *International Journal of Food Microbiology*, 141(1-2), pp.56–62.
- Hirshfield, I.N., Terzulli, S. and O’Byrne, C. (2003). Weak Organic Acids: A Panoply of Effects on Bacteria. *Science Progress*, 86(4), pp.245–270.
- Hmimsa, Y., Aumeeruddy-Thomas, Y. and Ater, M. (2017). Une forme spontanée de figuier (*Ficus carica* L.), le nābūt. *Revue d’ethnoécologie*, (Supplément 1).
- Ho, C.W., Lazim, A.M., Fazry, S., Zaki, U.K.H.H. and Lim, S.J. (2017a). Varieties, production, composition and health benefits of vinegars: A review. *Food chemistry*, [online] 221, pp.1621–1630. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/27979138>.
- Ho, W.C., Lazim, A.M., Fazry, S., Zaki, U.K.H.H. and Lim, S.J. (2017b). Effects of Fermentation Time and pH on Soursop (*Annona muricata*) Vinegar Production towards Its Chemical Compositions. *Sains Malaysiana*, [online] 46(9), pp.1505–1512. Available at: <https://core.ac.uk/download/pdf/154905647.pdf> [Accessed 18 Nov. 2021].
- Hussain, S.Z., Naseer, B., Qadri, T., Fatima, T. and Bhat, T.A. (2021a). Fig (*Ficus Carica*)—Morphology, Taxonomy, Composition and Health Benefits. *Fruits Grown in Highland Regions of the Himalayas*, pp.77–90.
- Hussain, S.Z., Naseer, B., Qadri, T., Fatima, T. and Bhat, T.A. (2021b). Grapes (*Vitis vinifera*)—Morphology, Taxonomy, Composition and Health Benefits. *Fruits Grown in Highland Regions of the Himalayas*, pp. 103-115.

- Ighbareyeh, J.M.H., Carmona, E.C., Ortiz, A.C., Suliemieh, A.A.-R.A., Ighbareyeh, M.M.H. and Daraweesh, A.A.-Q.M. (2018). Analysis of physical factors of climate and bioclimate and their effects on almonds production to increase the economy in Hebron area of Palestine. *Arabian Journal of Geosciences*, 11(21).
- Ilabaca, C., Navarrete, P., Mardones, P., Romero, J. and Mas, A. (2008). Application of culture culture-independent molecular biology-based methods to evaluate acetic acid bacteria diversity during vinegar processing. *International Journal of Food Microbiology*, 126(1-2), pp.245–249.
- Isham, N.K.M., Mokhtar, N., Fazry, S. and Lim, S.J. (2019). The development of an alternative fermentation model system for vinegar production. *LWT*, 100, pp.322–327.
- ISO 15214 (British Standard) (1998). Microbiology of food and animal feeding stuffs – Horizontal methods for enumeration of mesophilic lactic acid bacteria. Colony-count at 30 ° C.
- ISO 21527-2 (2008). Microbiology - General guidance for enumeration of yeasts and molds. Colony count technique at 25 ° C.
- ISO 4833-1 (2013). Microbiology of food and animal feeding stuffs - Horizontal methods for enumeration of microorganisms - Colony-count at 30 ° C.
- Jackson, R.S. (2008). *Wine science : principles and applications*. London: Academic.
- Jeong, W.-S. and Lachance, P.A. (2001). Phytosterols and Fatty Acids in Fig (*Ficus carica*, var. Mission) Fruit and Tree Components. *Journal of Food Science*, 66(2), pp.278–281.
- Ji-yong, S., Xiao-bo, Z., Xiao-wei, H., Jie-wen, Z., Yanxiao, L., Limin, H. and Jianchun, Z. (2013). Rapid detecting total acid content and classifying different types of vinegar based on near infrared spectroscopy and least-squares support vector machine. *Food Chemistry*, 138(1), pp.192–199.
- Jo, D., Kim, G.-R., Yeo, S.-H., Jeong, Y.-J., Noh, B.S. and Kwon, J.-H. (2013). Analysis of aroma compounds of commercial cider vinegars with different acidities using SPME/GC-MS, electronic nose, and sensory evaluation. *Food Science and Biotechnology*, 22(6), pp.1559–1565.

- Kadiroğlu, P. (2018). FTIR spectroscopy for prediction of quality parameters and antimicrobial activity of commercial vinegars with chemometrics. *Journal of the Science of Food and Agriculture*, 98(11), pp.4121–4127.
- Katz, S.H. and Weaver, W.W. (2003). *Encyclopedia of food and culture*. 2nd ed. New York: Scribner.
- Kelebek, H., Kadiroğlu, P., Demircan, N.B. and Selli, S. (2017). Screening of bioactive components in grape and apple vinegars: Antioxidant and antimicrobial potential. *Journal of the Institute of Brewing*, 123(3), pp.407–416.
- Kerstens, K., Lisdiyanti, P., Komagata, K. and Swings, J. (2006). The Family Acetobacteraceae: The Genera Acetobacter, Acidomonas, Asaia, Gluconacetobacter, Gluconobacter, and Kozakia. *The Prokaryotes*, [online] pp.163–200. Available at: [https://link.springer.com/referenceworkentry/10.1007%2F0-387-30745-1\\_9](https://link.springer.com/referenceworkentry/10.1007%2F0-387-30745-1_9) [Accessed 20 Dec. 2020].
- Khadivi, A., Anjam, R. and Anjam, K. (2018). Morphological and pomological characterization of edible fig (*Ficus carica* L.) to select the superior trees. *Scientia Horticulturae*, 238, pp.66–74.
- Kim, D.-H., Chon, J.-W., Kim, H. and Seo, K.-H. (2019). Development of a novel selective medium for the isolation and enumeration of acetic acid bacteria from various foods. *Food Control*, 106, p.106717.
- Kong, M., Lampinen, B., Shackel, K. and Crisosto, C.H. (2013). Fruit skin side cracking and ostiole-end splitting shorten postharvest life in fresh figs (*Ficus carica* L.), but are reduced by deficit irrigation. *Postharvest Biology and Technology*, 85, pp.154–161.
- Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E. and Etherton, T.D. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *The American Journal of Medicine*, 113(9), pp.71–88.
- La China, S., Zanichelli, G., De Vero, L. and Gullo, M. (2018). Oxidative fermentations and exopolysaccharides production by acetic acid bacteria: a mini review. *Biotechnology Letters*, 40(9-10), pp.1289–1302.
- Lansky, E.P., Paavilainen, H.M., Pawlus, A.D. and Newman, R.A. (2008). *Ficus* spp. (fig): Ethnobotany and potential as anticancer and anti-inflammatory agents. *Journal of Ethnopharmacology*, [online] 119(2), pp.195–213. Available at:

[https://www.sciencedirect.com/science/article/pii/S0378874108003309?casa\\_token=-  
tUsDiRQZR0AAAAA:rLb5IF-  
xaYZqriYW13jXnVrmUxj9rSDXUP1OiPkyijECDEAWfs5IDSmYNjaq4gZeazNayfHrgfY](https://www.sciencedirect.com/science/article/pii/S0378874108003309?casa_token=-tUsDiRQZR0AAAAA:rLb5IF-<br/>xaYZqriYW13jXnVrmUxj9rSDXUP1OiPkyijECDEAWfs5IDSmYNjaq4gZeazNayfHrgfY)  
[Accessed 5 Oct. 2020].

- Lebot, V. (2020). *Tropical root and tuber crops: cassava, sweet potato, yams and aroids*. Wallingford, Oxfordshire, Uk; Boston, Ma: Cabl.
- Li, S., Li, P., Feng, F. and Luo, L.-X. (2015). Microbial diversity and their roles in the vinegar fermentation process. *Applied Microbiology and Biotechnology*, [online] 99(12), pp.4997–5024. Available at: <https://link.springer.com/article/10.1007%2Fs00253-015-6659-1>.
- Liu, R.H. (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *The American Journal of Clinical Nutrition*, [online] 78(3), pp.517S520S. Available at: <https://academic.oup.com/ajcn/article/78/3/517S/4689990>.
- Liu, Y.-P., Guo, J.-M., Yan, G., Zhang, M.-M., Zhang, W.-H., Qiang, L. and Fu, Y.-H. (2019). Anti-Inflammatory and Antiproliferative Prenylated Isoflavone Derivatives from the Fruits of *Ficus carica*. *Journal of Agricultural and Food Chemistry*, 67(17), pp.4817–4823.
- Lo Turco, V., Potortì, A.G., Tropea, A., Dugo, G. and Di Bella, G. (2020). Element analysis of dried figs (*Ficus carica* L.) from the Mediterranean areas. *Journal of Food Composition and Analysis*, 90, p.103503.
- López, F., Pescador, P., Güell, C., Morales, M.L., García-Parrilla, M.C. and Troncoso, A.M. (2005). Industrial vinegar clarification by cross-flow microfiltration: effect on colour and polyphenol content. *Journal of Food Engineering*, 68(1), pp.133–136.
- Lynch, K.M., Zannini, E., Wilkinson, S., Daenen, L. and 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(3), pp.587–625.
- Mallikarjunan, P. and Mittal, G.S. (1994). Color Kinetics During Beef Carcass Chilling. *Transactions of the ASAE*, 37(1), pp.203–209.
- Mamlouk, D. and Gullo, M. (2013). Acetic Acid Bacteria: Physiology and Carbon Sources Oxidation. *Indian Journal of Microbiology*, [online] 53(4), pp.377–384. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3779290/>.

- Manzocco, L., Alongi, M., Sillani, S. and Nicoli, M.C. (2016). Technological and Consumer Strategies to Tackle Food Wasting. *Food Engineering Reviews*, 8(4), pp.457–467.
- Mas, A., Torija, M.J., García-Parrilla, M. del C. and Troncoso, A.M. (2014). Acetic acid bacteria and the Production and Quality of Wine Vinegar. *The Scientific World Journal*, [online] 2014, pp.1–6. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3918346/> [Accessed 16 Oct. 2019].
- Matallana, E. and Aranda, A. (2016). Biotechnological impact of stress response on wine yeast. *Letters in Applied Microbiology*, 64(2), pp.103–110.
- Matsushita, K., Toyama, H., Tonouchi, N. and Okamoto-Kainuma, A. (2016). *Acetic Acid Bacteria Ecology and Physiology*. 1st ed. Springer, Tokyo, pp.159–178.
- Mazza, S. and Murooka, Y. (2009). Vinegars Through the Ages. *Vinegars of the World*, pp.17–39.
- Molelekoa, T.J., Regnier, T., da Silva, L.S. and Augustyn, W.A. (2018). Potential of marula (*Sclerocarya birrea* subsp. *caffra*) waste for the production of vinegar through surface and submerged fermentation. *South African Journal of Science*, 114(11/12).
- Nahler, G. (2013). *Dictionary of Pharmaceutical Medicine*. Vienna Springer Vienna.
- Nanda, K., Taniguchi, M., Ujike, S., Ishihara, N., Mori, H., Ono, H. and Murooka, Y. (2001). Characterization of Acetic acid bacteria in Traditional Acetic Acid Fermentation of Rice Vinegar (Komesu) and Unpolished Rice Vinegar (Kurosu) Produced in Japan. *Applied and Environmental Microbiology*, 67(2), pp.986–990.
- National Committee for Clinical Laboratory Standards (1997). Performance standards for antimicrobial disk susceptibility test (6th ed.). Approved Standard. M2-A6: Wayne PA. Substitute by the European reference.
- Official Journal of the European Union (OJEU) (2015) COMMISSION IMPLEMENTING REGULATION (EU) 2015/48 of 14 January 2015 entering a name in the register of protected designations of origin and protected geographical indications [Vinagre de Montilla-Moriles (PDO)]. OJ L 9, 15.1.2015, pp 11–16

- Osada, K., Suzuki, T., Kawakami, Y., Senda, M., Kasai, A., Sami, M., Ohta, Y., Kanda, T. and Ikeda, M. (2006). Dose-dependent hypocholesterolemic actions of dietary apple polyphenol in rats fed cholesterol. *Lipids*, 41(2), pp.133–139.
- Osuala, O.J., Ezemba, C.C., Chude, C.O., Ezemba, A.S. and Anaukwu, C. (2021). Antimicrobial Analysis of Traditional and Industrial Produced Vinegar. *International Journal of Biosciences and Technology*, 14(3), pp.28–43.
- Ozturk, I., Caliskan, O., Tornuk, F., Ozcan, N., Yalcin, H., Baslar, M. and Sagdic, O. (2015). Antioxidant, antimicrobial, mineral, volatile, physicochemical and microbiological characteristics of traditional home-made Turkish vinegars. *LWT - Food Science and Technology*, 63(1), pp.144–151.
- P, V., Dash, S.K. and Rayaguru, K. (2019). Post-Harvest Processing and Utilization of Sweet Potato: A Review. *Food Reviews International*, 35(8), pp.726–762.
- Panda, S.K., Mishra, S.S., Kayitesi, E. and Ray, R.C. (2016). Microbial-processing of fruit and vegetable wastes for production of vital enzymes and organic acids: Biotechnology and scopes. *Environmental Research*, [online] 146, pp.161–172. Available at: <https://www.sciencedirect.com/science/article/pii/S0013935115301973>.
- Pandey, K.B. and Rizvi, S.I. (2009). Plant Polyphenols as Dietary Antioxidants in Human Health and Disease. *Oxidative Medicine and Cellular Longevity*, [online] 2(5), pp.270–278. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2835915/>.
- Papargyropoulou, E., Wright, N., Lozano, R., Steinberger, J., Padfield, R. and Ujang, Z. (2016). Conceptual framework for the study of food waste generation and prevention in the hospitality sector. *Waste Management*, 49, pp.326–336.
- Parapouli, M., Vasileiadi, A., Afendra, A.-S. and Hatziloukas, E. (2020). *Saccharomyces cerevisiae* and its industrial applications. *AIMS Microbiology*, [online] 6(1), pp.1–32. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7099199/>.
- Peng, W., Ma, Q., Wang, Z. and Xie, Z. (2019). Research Progress On Comprehensive Utilization Of Fruit And Vegetable Waste. *E3S Web of Conferences*, 131, p.01106.
- Plazzotta, S., Manzocco, L. and Nicoli, M.C. (2017). Fruit and vegetable waste management and the challenge of fresh-cut salad. *Trends in Food Science & Technology*, 63, pp.51–59.

- Prior, R.L. and Cao, G. (2000). Flavonoids: Diet and Health Relationships. *Nutrition in Clinical Care*, 3(5), pp.279–288.
- Qiu, X., Zhang, Y. and Hong, H. (2021). Classification of acetic acid bacteria and their acid resistant mechanism. *AMB Express*, 11(1).
- Ramos, B., Brandão, T.R.S., Teixeira, P. and Silva, C.L.M. (2014). Balsamic vinegar from Modena: An easy and effective approach to reduce *Listeria monocytogenes* from lettuce. *Food Control*, 42, pp.38–42.
- Raspor, P. and Goranovic, D. (2008). Biotechnological applications of acetic acid bacteria. *Critical reviews in biotechnology*, [online] 28(2), pp.101–24. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/18568850>.
- Ríos-Reina, R., Callejón, R.M., Oliver-Pozo, C., Amigo, J.M. and García-González, D.L. (2017). ATR-FTIR as a potential tool for controlling high quality vinegar categories. *Food Control*, 78, pp.230–237.
- Ríos-Reina, R., García-González, D.L., Callejón, R.M. and Amigo, J.M. (2018). NIR spectroscopy and chemometrics for the typification of Spanish wine vinegars with a protected designation of origin. *Food control*, 89(1), pp.108–116.
- Roda, A., Lucini, L., Torchio, F., Dordoni, R., De Faveri, D.M. and Lambri, M. (2017). Metabolite profiling and volatiles of pineapple wine and vinegar obtained from pineapple waste. *Food Chemistry*, 229, pp.734–742.
- Rogers, P., Chen, J.-S. and Zidwick, M.J. (2013). Organic Acid and Solvent Production: Acetic, Lactic, Gluconic, Succinic, and Polyhydroxyalkanoic Acids. *The Prokaryotes*, pp.3–75.
- Roullier-Gall, C., David, V., Hemmler, D., Schmitt-Kopplin, P. and Alexandre, H. (2020). Exploring yeast interactions through metabolic profiling. *Scientific Reports*, [online] 10(1), p.6073. Available at: <https://www.nature.com/articles/s41598-020-63182-6> [Accessed 6 Oct. 2021].
- Sagar, N.A., Pareek, S., Sharma, S., Yahia, E.M. and Lobo, M.G. (2018). Fruit and Vegetable Waste: Bioactive Compounds, Their Extraction, and Possible Utilization. *Comprehensive Reviews in Food Science and Food Safety*, 17(3), pp.512–531.

- Saha, P. and Banerjee, S. (2013). Optimization of process parameters for vinegar production using banana fermentation. *International Journal of Research in Engineering and Technology*, 2(9), pp.501–514.
- Saichana, N., Matsushita, K., Adachi, O., Frébort, I. and Frebortova, J. (2015). Acetic acid bacteria: A group of bacteria with versatile biotechnological applications. *Biotechnology Advances*, [online] 33(6), pp.1260–1271. Available at: <https://www.sciencedirect.com/science/article/pii/S0734975014001888>.
- Santo, D.E., Galego, L., Gonçalves, T. and Quintas, C. (2012). Yeast diversity in the Mediterranean strawberry tree (*Arbutus unedo* L.) fruits' fermentations. *Food Research International*, 47(1), pp.45–50.
- Scalbert, A., Manach, C., Morand, C., Rémésy, C. and Jiménez, L. (2005). Dietary Polyphenols and the Prevention of Diseases. *Critical Reviews in Food Science and Nutrition*, 45(4), pp.287–306.
- Sengun, I.Y. (2013). Microbiological and chemical properties of fig vinegar produced in Turkey. *African Journal of Microbiology Research*, 7(20), pp.2332–2338.
- Sengun, I.Y. (2017). *Acetic acid bacteria: Fundamentals and Food Applications*. Boca Raton, FL: Crc Press.
- Sengun, I.Y., Kilic, G. and Ozturk, B. (2019). Screening physicochemical, microbiological and bioactive properties of fruit vinegars produced from various raw materials. *Food Science and Biotechnology*, 29(3), pp.401–408.
- Solieri, L. and Giudici, P. (2009). *Vinegars of the World*. Milano Springer Milan.
- Solomon, A., Golubowicz, S., Yablowicz, Z., Grossman, S., Bergman, M., Gottlieb, H.E., Altman, A., Kerem, Z. and Flaishman, M.A. (2006). Antioxidant Activities and Anthocyanin Content of Fresh Fruits of Common Fig (*Ficus carica* L.). *Journal of Agricultural and Food Chemistry*, 54(20), pp.7717–7723.
- Sousa, L.L., Andrade, S.C.A., Athayde, A.J.A.A., Oliveira, C.E.V., Sales, C.V., Madruga, M.S. and Souza, E.L. (2013). Efficacy of *Origanum vulgare* L. and *Rosmarinus officinalis* L. essential oils in combination to control postharvest pathogenic *Aspergilli* and autochthonous mycoflora in *Vitis labrusca* L. (table grapes). *International Journal of Food Microbiology*, [online]

165(3), pp.312–318. Available at:  
<https://www.sciencedirect.com/science/article/pii/S0168160513002754>.

Sun, X., Wang, Z., Kadouh, H. and Zhou, K. (2014). The antimicrobial, mechanical, physical and structural properties of chitosan–gallic acid films. *LWT - Food Science and Technology*, 57(1), pp.83–89.

Szalay, J. (2018). *Sweet Potatoes: Delicious and Nutritious*. [online] Live Science. Available at:  
<https://www.livescience.com/46016-sweet-potato-nutrition.html>.

Tan, S.C. (2005). *Vinegar fermentation*. MSc Thesis.

Tanamool, V., Chantarangsee, M. and Soemphol, W. (2020). Simultaneous vinegar fermentation from a pineapple by-product using the co-inoculation of yeast and thermotolerant acetic acid bacteria and their physiochemical properties. *3 Biotech*, 10(3).

Tesfaye, W., Garcia-Parrilla, M. C. and Troncoso, A. M. (2002a). Sensory evaluation of sherry wine vinegar. *Journal of Sensory Studies*, 17(2), pp.133-144.

Tesfaye, W., García-Parrilla, M.C. and Troncoso, A.M. (2000). Set Up and Optimization of a Laboratory Scale Fermentor for the Production of Wine Vinegar. *Journal of the Institute of Brewing*, 106(4), pp.215–220.

Tesfaye, W., Morales, M.L., García-Parrilla, M.C. and Troncoso, A.M. (2003). Optimising wine vinegar production: fermentation and ageing. *Applied Biotechnology, Food Science and Policy*, 1(2), pp.109-114.

Tesfaye, W., Morales, M.L., García-Parrilla, M.C. and Troncoso, A.M. (2002b). Wine vinegar: technology, authenticity and quality evaluation. *Trends in Food Science & Technology*, 13(1), pp.12–21.

Trček, J. and Barja, F. (2015). Updates on quick identification of acetic acid bacteria with a focus on the 16S–23S rRNA gene internal transcribed spacer and the analysis of cell proteins by MALDI-TOF mass spectrometry. *International Journal of Food Microbiology*, 196, pp.137–144.

- Tumane P.M, Sarkar S, Wasnik D.D. and Kolte N.A (2018). Production of vinegar from pineapple peels using *Acetobacter* species isolated from soil sample and its antibacterial activity. *International Journal of Life Sciences*, 6(4), pp.948-956.
- Ubeda, C., Callejón, R.M., Hidalgo, C., Torija, M.J., Mas, A., Troncoso, A.M. and Morales, M.L. (2011). Determination of major volatile compounds during the production of fruit vinegars by static headspace gas chromatography–mass spectrometry method. *Food Research International*, 44(1), pp.259–268.
- Valero, E., Berlanga, T.M., Roldán, P.M., Jiménez, C., García, I. and Mauricio, J.C. (2005). Free amino acids and volatile compounds in vinegars obtained from different types of substrate. *Journal of the Science of Food and Agriculture*, 85(4), pp.603–608.
- Veberic, R. and Mikulic-Petkovsek, M. (2016). Phytochemical Composition of Common Fig (*Ficus carica* L.) Cultivars. *Nutritional Composition of Fruit Cultivars*, pp.235–255.
- Vidra, A. and Németh, Á. (2017). Bio-produced Acetic Acid: A Review. *Periodica Polytechnica Chemical Engineering*, 62(3), pp.245–256.
- Wang, A., Li, R., Ren, L., Gao, X., Zhang, Y., Ma, Z., Ma, D. and Luo, Y. (2018). A comparative metabolomics study of flavonoids in sweet potato with different flesh colors (*Ipomoea batatas* (L.) Lam). *Food Chemistry*, 260, pp.124–134.
- Yahia, E.M. (2017). *Fruit and Vegetable Phytochemicals Chemistry and Human Health, 2nd Edition*. Chichester, Uk John Wiley & Sons, Ltd.
- Yamada, Y. (2016). Systematics of Acetic Acid Bacteria. *Acetic Acid Bacteria*, pp.1–50.
- Yang, X., Yu, W., Ou, Z., Ma, H., Liu, W. and Ji, X. (2009). Antioxidant and Immunity Activity of Water Extract and Crude Polysaccharide from *Ficus carica* L. Fruit. *Plant Foods for Human Nutrition*, 64(2), pp.167–173.
- Zolfaghari, M.R., Salimpour, F., Sharifinia, F., Peyvandi, M. and Jafari Marandi, S. (2019). Intraspecific morphological and genetic variations of *Ficus carica* L. from Iran. *Biologija*, 65(3), pp.209–220.

Zou B, Dong X, Ge Z, Xu Z, Du J, Li C. Development of suitable standards for quantitative determination of persimmon phenol contents in Folin–Ciocalteu and vanillin assays. *Eur. Food Res. Technol* 239: 385–391 (2014).