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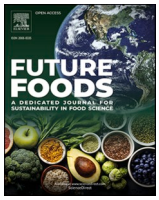

Quality and biological properties of vinegar processed from non-valorized fruits in Southern Portugal

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ABSTRACT

The processing of vinegar from non-valorized fruits and agricultural surpluses is an attractive strategy for biomass waste management. The present study aimed to characterize vinegar of fermented fruits, namely fresh fig, plum, dried fig, grape, raspberry, and apricot. Physicochemical and microbial quality, total phenolic content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC), mineral composition, and antimicrobial capacity against bacteria and yeasts were studied. Grape and apricot vinegar had the highest values of TAC (39.31±0.25, 34.10±1.44 DPPH μg Trolox/100 mL, respectively), while grape contained the highest TFC (35.73 ±3.86 mg catechin/100 mL) and raspberry and fresh fig, the highest TPC (55.40±1.1, 64.10±0.0 mg gallic acid/ 100 mL, respectively). Potassium, calcium and sodium were the most abundant minerals in the vinegars with dried fig standing out for its richness in potassium 1223.83±89.48 mg/L, calcium 17.70±1.31 mg/L, magnesium 2.39±0.24 mg/L, and zinc 4.33±0.17 mg/L. The enterobacteria *Cronobacter sakazakii* and *Salmonella* Typhimurium were the most tolerant (10.00±0.00–26.39±1.60 mm), while *Pseudomonas aeruginosa* was the most sensitive bacteria (26.36±3.61–40.17±2.72 mm) as well as Gram + (*Staphylococcus aureus, Listeria innocua*) (21,20±8,37–31.11±2.41 mm). The acetification processes resulted in vinegars with a good hygienic status, and levels of acetic acid (≥5 %) complying with the legislation.

Introduction

The term vinegar comes from the French word “vinaigre”, signifying “sour wine”, which results from the acetification of wine, a fermented beverage processed in the Mediterranean areas where vineyards were cultivated (Bourgeois and Barja, 2009). Nowadays, vinegar is manufactured worldwide using various materials rich in carbohydrates, mainly fruits, in Western countries and cereals, in Asian regions. Fruit vinegar is produced from grape, apple, pineapple, mango, plum, banana, orange, cherry, among others, and cereal vinegar is obtained from rice, sorghum, wheat, barley, malt, as well as other grains rich in starch (Solieri and Giudici, 2009; Kawa-Rygielska et al., 2018; Xie et al., 2022). In addition, non-conventional sources, such as by-products (whey) of other industries (dairy industry), non-expensive raw materials, non-valorized and surplus materials, available in different geographical regions may also be used to produce acetified products (Solieri and Giudici, 2009). In industrialized countries, the overproduction, post-harvest losses, and non-compliance with retailer/consumer requirements of fruits and vegetables is a serious environmental problem and has a significant economic impact (Plazzotta et al., 2017; Panda et al., 2016). As a result, much attention has been given in recent years to creating policies for the management and implementation of strategies for waste transformation into valuable resources (Esparza et al., 2020; Luzón-Quintana et al., 2021).

Vinegar is produced in two biological processes that begin with the alcoholic fermentation of carbohydrates where yeasts consume organic molecules to obtain energy (ATP), ethanol, CO_2, and reducing power in the absence of oxygen, and ends with acetification, a process performed by acetic acid bacteria (AAB), obligate aerobes, that incompletely oxidize ethanol into acetic acid with oxygen as the final electrons' acceptor (Vegas et al., 2010; Qiu et al., 2021; He et al., 2022). Acetification may occur in French acetifiers (Orleans method, slow method),

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German rapid acetifiers (Rapid method) and submerged acetifiers. In the first system the AAB are in the "mother of vinegar", a film of AAB placed at the liquid-gas interface in contact with air, in the second method the AAB are immobilized in the wood shavings (or other material) located in the acetifier and the liquid is permanently pumped over the shavings to increase oxygen supply. Finally, in the latter, the AAB are submerged in the liquid to acetify and air is continuously introduced through compressors (Ho et al., 2017). Acetic acid defines the main organoleptic properties of vinegar and its content in wine vinegar should not be below 60 g/L (w/v) and in other vinegars (fruit) below 50 g/L, according to the Portuguese legislation (Decreto-Lei nº 174/2007). In addition to acetic acid, vinegars contain other fermentation compounds, such as esters, ketones and aldehydes, which are associated with flavor and color, and also contain substances with biological properties (antimicrobial and antioxidants), as well as minerals (Na, K, Ca, Mg, Zn). Due to their properties, vinegars possess very different applications, namely seasoning, aromatization, preservation, therapeutics, and antimicrobial (antifungal and antibacterial), with references to their use in the food industry and traditional medicine (Budak et al., 2014; Luzón-Quintana et al., 2021). A randomized trial showed that vinegar, ingested daily, has an anti-calcium oxalate (CaOx) crystals development suppressing the development of stones in the kidneys (Zhu et al., 2019).

The objective of this work was to study the characteristics of different vinegars processed in southern Portugal (Algarve). The vinegars were produced from non-valorized fruits (substandard fruit) and agricultural surpluses. As a result, it is important to study the properties of the final products to evaluate their compliance with the legislation, namely, their physicochemical and microbial properties. The specific objectives of the study were to evaluate the physiochemical (pH, total acidity, °Brix, color parameters, minerals (Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Manganese (Mn) and Zink (Zn)) and microbial properties (total microbial counts, lactic acid bacteria (LAB), acetic acid bacteria (AAB) and filamentous fungi and yeasts) of the vinegars as well as to determine the bioactive properties, namely antioxidant and antimicrobial properties.

Materials and methods

The types of vinegar studied in the present work were processed in a small company in the Algarve region (Algoz) using the Orleans method and marketed under the brand name "Fateixa" in glass bottles of 250 mL. Six different fruit vinegar with the labeled acidity values, indicated in percentage, were collected (in sextuples): fresh fig (5 %), plum (5 %), dried fig (6 %), grape (6 %), raspberry (7 %) and apricot (7 %). The fruit used in the manufacture of vinegar was produced on local farms in Southern Portugal (Algarve). Ripe and over-ripe fruit (not spoiled and suitable for human consumption), substandard, possessing defects in shape, color, and texture, and not conforming with commercialization criteria, were selected. Dried fruits were used in the case of dried fig vinegar. Fruits with any signs of deterioration (fungal, insect) were rejected.

Fresh fruit was crushed (250 kg–500 kg) in stainless steel vessels, and inoculated with yeast (*Saccharomyces cerevisiae)* (EnartisFerm WS, Trecate, Italy) where an alcoholic fermentation occurred, at room temperature, until the obtention of ethanol levels of 7 %–10 % (v/v) and ºBrix contents of 4,5–8. Then, the alcoholic musts were filtered using a stainless-steel sieve (0,5 mm) and transferred to French oak barrels (225 L) where acetification occurred, as a result of the natural formation of the "mother of vinegar" at the air-liquid interface in contact with oxygen. The acetification processes required long periods and were considered finished when the acetified product possessed the desired organoleptic characteristics. This phase could last 8–12 months to be finished. Subsequently, the acetous liquid was discharged into stainless-steel vessels where clarification occurred by sedimentation. After 3–6 months the products were filtered (cellulose filter, 10 μm), pasteurized (60 ºC, 1h30 min), packed in 250 mL bottles, and labeled.

The reagents used in the physicochemical and microbial analyses were analytical grade and bought from different commercial sources.

Physicochemical quality

pH

The pH measurement was taken using a pH-Meter (Crison, Model Basic 20, Barcelona, Spain) with a glass electrode and an automatic paired temperature sensor, previously calibrated. Measurements were taken in triplicate and averages and standard deviation were reported.

Titratable acidity

The total titratable acidity (TA) was determined by titrating the vinegars with 0.1 M NaOH (Rhône-Poulenc, Paris, France) using phenolphthalein (2 %) (Merck, Darmstadt, Germany) as an indicator and the results were expressed in g of acetic acid per 100 mL (%). Experiments were conducted in triplicate and averages and standard deviation were reported.

Total soluble solids (°Brix)

Brix values of the vinegars were measured using an Abbe refractometer (Atago, Saitama, Japan) calibrated with distilled water. The values were expressed as °Brix. Measurements were taken in triplicate and average and standard deviation were reported.

Color

The color of the vinegars was measured using a handheld colorimeter (PCE-CSM 10, PCE Instruments spectrophotometer, Meschede-Freienohl, Germany) and was represented by the L* a* b* color notation, which is a 3-D color presentation method where 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 placed on a sample holder where color was measured. Averages of five measurements and standard deviation were recorded in different portions of each sample.

Total phenolic content

The total phenolic content (TPC) was analyzed based on the Folin–Ciocalteu method (Zou et al., 2014) with some modifications and using gallic acid (98 %) (Acros Organics, Geel, Belgium) (0.0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0 mg/100 mL of gallic acid) to obtain a standard calibration curve. In brief, the appropriate dilution of vinegar (0.2 mL) was combined with 1 mL of Folin–Ciocalteu reagent (0.2 N) (Merck, Darmstadt, Germany). Then, 0.8 mL of Na_2CO_3 (7.5 %, w/v) (Merck, Darmstadt, Germany) was added to the mixture and after the proper shaking, the solution was allowed to stand at room temperature in the dark for 30 min. The absorbance of the solution at 765 nm was measured with a spectrophotometer (Spectronic GENESYS 10, Madison, Wisconsin, USA) and the results were expressed in mg of gallic acid equivalent (GAE) per 100 mL (mg GAE/100 mL). The TPC was calculated using the calibration curve equation obtained (ABS_{765nm}=7.68[GAE]+0.019, R^2=0.995). Experiments were conducted in triplicate and average and standard deviation were reported.

Total flavonoid content

The total flavonoid content (TFC) was determined based on 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 the addition of 0.3 mL of a 5 % $NaNO_2$ (v/v) (Merck, Darmstadt, Germany) and vigorously mixed in a vortex. The mixture was kept in the dark at room temperature. After 5 min, 0.3 mL of a 2 % $AlCl_3$ (w/v) (Riedel-de Haën, Seelze, Germany) was

added, shaken (using a vortex), and allowed to stand for 6 min in the dark. After, 2 mL of 1 M NaOH (Rhône-Poulenc) were 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 using a spectrophotometer (Spectronic GENESYS 10) in comparison with the standards prepared similarly with (+)-catechin (TCI, Zwijndrecht, Belgium) (0.0, 1.0, 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 mg/100 mL). The TFC was calculated using the calibration curve equation ($ABS_{510\ nm}$=0.0022 [catechin]+0.0079, R^2=0.996) and expressed as mg of catechin equivalents per 100 mL of vinegar (mg catechin equivalent/100 mL). Experiments were conducted in triplicate and averages and standard deviations were reported.

Total antioxidant capacity

The total antioxidant activity (TAC) of the vinegars, based on the scavenging activity of the stable 1,1-diphenyl-2- picrylhydrazyl (DPPH) (Sigma-Aldrich, Steinhem, Germany) free radical, was determined by the method described by Braca et al. (2001). The vinegar samples (0.1 mL) were combined with 5 mL of a 0.1 mM DPPH (Sigma-Aldrich) in test tubes and vigorously mixed using a vortex. Methanol (MeOH) (0.1 mL) (VWR, Fontenay-sous-Bois, France) was used as a control. Following an incubation of 13 min, in a bath at 27 ºC in the dark, absorbance was measured at 515 nm, against methanol, using a spectrophotometer (Spectronic GENESYS 10). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) ($C_{14}H_{18}O_4$) (Sigma-Aldrich, Steinhem, Germany) (8, 10, 15, 20, 25, 30, 35 and 40 μg TE/100 mL), was used to obtain a standard curve equation (ABS_{515nm}=–0.45[Trolox]+0.56, R^2=0.998) in which the TAC of the vinegar samples was calculated and expressed as μg of Trolox equivalents per 100 mL (μg TE/100 mL). Experiments were conducted in triplicates and averages and standard deviation were reported.

Minerals

The determination of Na, K, Ca, Mg, Mn and Zn contents was done by flame atomic absorption spectrophotometry (F-AAS). Triplicates of 25 mL aliquots of each vinegar were taken from the homogenized samples into 50 mL polyethylene digestion flasks (SCP Science). To each aliquot 5 mL of HNO_3 (67 %, v/v) (VWR, Normaton, UK) was added and digestion was carried out in a fume hood in an open system controlled by reflux caps, at 800 ºC for two and a half hours. Then, the reflux covers were removed and evaporation was maintained at 800 ºC until a final volume of approximately 5 mL was reached. After cooling, the volume was brought up to 25 mL with 0.2 % HNO_3 (v/v) (VWR). Na, K, Ca, Mg, Mn and Zn were then analyzed by air-acetylene flame atomic absorption spectrophotometry, using a spectrophotometer (Analytik Jena Nova 800, Jena, Germany) according to the specific programs for each metal and the necessary adjustments to minimize interferences of the matrix for Na, K, Ca and Mg. All metals were determined based on chemical calibrations performed using calibration curves prepared from commercially available concentrated metal solutions (1000 mg/L). The calibration curves were validated using internal standards from another source. Recovery tests were also carried out in the digestion process and blank tests. The content of minerals was expressed in mg/mL. Experiments were conducted in triplicates and averages and standard deviation were reported.

Microbiological quality

The evaluation of the microbiological quality [aerobic microorganisms at 30 ºC, filamentous fungi, yeasts, acetic acid bacteria, and lactic acid bacteria] of the different products was carried out in three bottles of each type of vinegar. For each analysis, after homogenization of the content of the bottles, samples were aseptically withdrawn, inoculated in the adequate culture medium, and incubated accordingly. The analyses were done in duplicate for each vinegar bottle.

Aerobic microorganisms were determined on Plate Count Agar (PCA) (Scharlau, Barcelona, Spain), pH 5.5, and incubated, for 3 days at 30 °C (ISO 4833–1, 2013). For the aerobic microorganism analysis, 1 mL of each bottle was incorporated in duplicate in PCA. MRS agar medium (Scharlau) incubated during 3–5 days at 30 °C (ISO 15214, British Standard, 1998) was used to culture Lactic-Acid Bacteria (LAB). For the LAB analysis, 1 mL of each bottle was incorporated, in duplicate, in the culture media. Filamentous fungi were cultured on Dicloran Rose Bengal Chloramphenicol agar (Biolife, Milan, Italy) after an incubation of 5–7 days at 25 °C (ISO 21527–2, 2008). The presence of yeasts was determined on Malt Extract Agar (ME) (Scharlau) incubated for 3–5 days at 25 ºC. Enumeration of filamentous fungi and yeasts was done through the spread plating inoculation of 1 mL (0.2 mL aliquots in five plates) onto the surface of the adequate culture medium, and incubated. "AAB-selective agar" (pH 4.8) was selected to enumerate acetic acid bacteria (AAB) (Kim et al., 2019). The composition of "AAB-selective agar" (1 L) was: d-(+) Glucose 50 g (Scharlau), yeast extract 10 g (BIOKAR Diagnostics, Adana, Turkey), bromophenol blue 20 mg (BDH, Poole, England), bacteriological agar (JMVP, Sintra, Portugal) 20 g, 1 mL of glacial acetic acid (Merck, Darmstadt, Germany), 50 mL of ethanol 96 % v/v (PanReac, Barcelona, Spain), water 950 mL (Kim et al., 2019). The pH of the culture media was adjusted with a 1 M hydrochloric acid solution. All the plates were incubated in aerobic conditions except LAB.

Antimicrobial capacity

The antimicrobial capacity of the vinegars was evaluated against 15 microorganisms. The Gram + bacteria tested were *Bacillus cereus* ATCC 10876, *Listeria innocua* ATCC 33090, *Staphylococcus aureus* ATCC 25923, and *Staphylococcus epidermidis* ATCC 12228. The Gram - bacteria tested were *Cronobacter sakazakii* ATCC 29544, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella enterica* Typhimurium ATCC 14028. Seven yeasts were also tested: three human potential pathogens, *Candida albicans* ATCC 10231, *Candida albicans* ATCC 90028, and *Cryptococcus neoformans* YPO 186, and four food spoilage yeasts, *Saccharomyces cerevisiae* PYCC 3507, *Pichia membranaefaciens* PYCC 2489, *Zygosaccharomyces bailii* PYCC 4806 and *Debaryomyces hansenii* PYCC 2968. The method to evaluate the antimicrobial activity of the vinegars was adapted from the agar diffusion method described in the protocols of the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2000).

Bacterial samples were cultivated on plates with Mueller Hinton agar (MHA) (Himedia, Maharashtra, India) (pH 5.5) and yeasts were cultivated on YM (YM Agar) (Himedia, Maharashtra, India) (pH 5.5) 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 yeast). Thereafter, the suspensions of microorganisms 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.

Using sterile swabs, the bacteria and yeast suspensions were inoculated on the surface of the media in order to obtain confluent and even microbial growth across the entire plate surfaces. Then, the inoculated plates were allowed to dry for 5 min. Subsequently, sterile filter paper blank discs (6 mm in diameter, Liofilchem, Roseto degli Abruzzi, Italy), were placed onto the surface of the agar plates previously inoculated with the standard suspensions of microorganisms to be tested. Each disc was impregnated with 20 μL of the vinegars. As negative control, 20 μL of 0.85 % saline solution was used in a disc. Positive control in this study for bacterial assessments Chloramphenicol (30 μg/disc, Liofilchem, Roseto degli Abruzzi, Italy). In the case of yeast, positive control Nystatin (100 IU/disc, Liofilchem, Roseto degli Abruzzi, Italy). The discs impregnated with the antibacterial and antifungal agents were placed on the plates inoculated with the suspensions of each bacteria/yeast.

Table 1
Physicochemical properties of vinegar from Algarve region.

Vinegar samples	pH	Total acidity (%)	° Brix
Fresh fig	3.465 ± 0.007^{d}	4.822 ± 0.070^{a}	8.400 ± 0.566^{c}
Plum	3.070 ± 0.044^{b}	5.246 ± 0.167^{b}	6.450 ± 0.071^{a}
Dried fig	3.495 ± 0.043^{f}	5.74 ± 0.21^{c}	11.200 ± 0.283^{d}
Grape	2.833 ± 0.018^{a}	6.752 ± 0.169^{d}	4.900 ± 0.455^{a}
Raspberry	3.300 ± 0.000^{c}	6.820 ± 0.060^{d}	$5.500 \pm 0.000^{a,b}$
Apricot	3.227 ± 0.012^{c}	7.332 ± 0.053^{e}	5.133 ± 0.115^{a}

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

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 inhibition of microbial growth and the diameters of these clear zones (halos) were measured in mm and recorded as averages and standard deviation. Each assay was replicated three times.

Statistical analyses

The results obtained were expressed as the averages of various determination measurements (depending on the analytical technique) and the standard deviation. Analysis of variance (ANOVA) was done and the values were compared using the Tukey test. Differences were considered statistically significant at $p < 0.05$. Statistical analyses were done using SPSS statistical software version 24.0 (IBM SPSS Statistics 24.0).

Results and discussion

Physiochemical quality

The results of the physiochemical properties of the six different fruit vinegar samples (fresh fig, plum, dried fig, grape, raspberry and apricot) are shown in Tables 1–4. The data obtained in all the parameters evaluated showed a great variability, pointing to different qualities of the vinegars which is explained by the variability of the raw materials used.

The pH levels ranged from 2.83 to 3.49, with grape having the lowest values ($p < 0.05$) and fig vinegars having the highest values (3.46–3.49) ($p < 0.05$). The values measured are similar to those obtained by other authors [(Ozturk et al., (2015); Bakir et al., (2017), Hammouda et al. (2021)]. Regarding titratable acidity, the levels determined ranged between 4.82 % (w/v) and 7.33 % (w/v) of acetic acid, with fresh fig and plum (5.25 %) vinegars having the lowest values and apricot the highest. Significant differences were found among these samples in terms of total acidity level ($p < 0.05$). Based on the Portuguese Legislation (DL174/2007), non-wine vinegars should have at least 5 % acetic acid (w/v) and the acidity levels of all vinegar samples studied were higher than 5 %. However, some of the vinegars analyzed in the studies of Ozturk et al., (2015), Bakir et al., (2017) and Hammouda et al. (2021) did not reach 5 % acidity. In the present study, the total soluble solids (°Brix) of the samples varied from 4.90 to 11.20. The lowest value was measured in grape (4.90) while the highest value was in dry fig vinegar (11.20). The °Brix of plum, grape, raspberry and apricot vinegars were significantly lower than fig vinegar ($p < 0,05$). In the case of °Brix, Budak (2015), Ozturk et al., (2015) and Hammouda et al., (2021) reported values in the fruit vinegars ranging from 1.02 to 21.97. The °Brix values of the vinegar samples are related to the starting material used in the processing and during the alcoholic fermentation, sugars are consumed by yeasts, which contribute to the decreasing of soluble solids in the final products.

Table 2
Bioactive properties of vinegars from Algarve region.

Vinegar samples	TPC (mg GAE/100 mL)	TFC (mg Catechin equivalent/ 100 mL)	DPPH (μg TE/100 mL)
Fresh fig	64.1 ± 0.0^{e}	$9.37 \pm 0.58^{b,c}$	28.12 ± 1.41^{d}
Plum	$51.0 \pm 2.4^{d,e}$	$8.64 \pm 0.11^{b,c}$	$9.70 \pm 0.07^{b,c}$
Dried fig	$21.7 \pm 2.6^{a,b,c}$	31.52 ± 0.68^{e}	$5.34 \pm 0.51^{a,b}$
Grape	8.40 ± 3.5^{a}	35.73 ± 3.86^{e}	39.31 ± 0.25^{e}
Raspberry	55.40 ± 1.1^{e}	16.16 ± 1.12^{d}	28.53 ± 1.52^{d}
Apricot	$15.5 \pm 6.9^{a,b}$	$5.278 \pm 0.30^{a,b}$	$34.1 \pm 1.44^{d,e}$

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

Table 3
Color parameters of vinegars from Algarve region.

Vinegar samples	L*	a*	b*
Fresh fig	26.852 ± 0.204^{a}	0.483 ± 0.101^{c}	3.172 ± 0.105^{b}
Plum	28.490 ± 0.161^{d}	-0.165 ± 0.052^{a}	3.129 ± 0.106^{b}
Dried fig	25.467 ± 0.927^{a}	0.286 ± 0.097^{b}	2.011 ± 0.045^{a}
Grape	26.893 ± 0.131^{b}	0.444 ± 0.037^{c}	3.232 ± 0.108^{b}
Raspberry	27.711 ± 0.236^{c}	0.199 ± 0.136^{b}	3.261 ± 0.154^{b}
Apricot	30.389 ± 0.078^{e}	-0.029 ± 0.144^{a}	$2.610 \pm 2.255^{a,b}$

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.

Total phenolic content, total flavonoid content and total antioxidant capacity

The total phenolic content (TPC), total flavonoid content (TFC) and antioxidant capacity (TAC) of the six different vinegar samples were determined by spectrophotometric methods, and the results obtained are summarized in Table 2. TPC ranged from 8.40 mg GAE/100 mL to 64.1 mg GAE/100 mL, while the highest content was found in the fresh fig vinegar, and the lowest was found in the grape vinegar. In general, fresh fig, plum, and raspberry vinegars TPC were significantly higher than the content obtained for the rest of the products ($p < 0.05$). The TPC determined in the present study, although different, are in the same range of the values found by Sengun et al. 2020 (grape 1025, apricot 1005, plum 1057, fig 935.5 mg GAE/L) and Hammouda et al. 2021 (grape 286.36 mg GAE/L; fig 1242.17 mg GAE/L). However, Cornelian cherry vinegar possessed higher TPC than the values obtained in the present study (Kawa-Kygieska et al., 2018). Regarding the TFC of the vinegars, samples were in the range of 8.64–35.73 mg catechin/100 mL (Table 2). Among the samples, the dried fig and grape vinegars exhibited significantly higher contents ($p < 0.05$) than the others, with apricot being the sample with the lowest level of TFC. The values obtained for TFC, in the current study are in the same range as those of Sengun et al. (2020) (grape 221 mg catechin/L, apricot 166 mg/L, plum 470 mg/L, fig 178 mg/L) and Hammouda et al. (2021) (grape 0.241 mg/L and fig 755 mg/L). The TAC of the vinegars was determined based on the scavenging activity of the stable 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical. This property varied from 5.34 μg TE/100 mL to 39.31 μg TE/100 mL, with the dried fig having the lowest level followed by plum vinegar when compared with grape and apricot ($p < 0.05$), which have the highest levels, followed by fresh fig and raspberry vinegars. The highest TAC was associated with the grape vinegar which was also one of the highest observed by Ozturk et al., (2015). However, the results of TAC obtained by Sengun et al., (2020) for grape (0.119 μg TE/mL), apricot (0.1302 μg TE/mL), plum (0.302 μg TE/mL), and fig (0.047 μg TE/mL) are different from the ones obtained in the present work, except dried fig. Similar to TPC and TFC, the TAC varied among the samples due to the substrates used and the processing methods applied as described by Ozturk et al., (2015), Kelebek et al., (2017), Cruz et al., (2018), Sengun et al., (2020) and Hammouda et al., (2021). The antioxidant potential of

Table 4
Mineral composition of vinegars from Algarve region.

Vinegar samples	Na (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	Mn (mg/L)	Zn (mg/L)
Fresh fig	$11.17 \pm 0.63^{c,d}$	681.33 ± 25.15^{d}	$12.14 \pm 0.97^{c,d}$	1.24 ± 0.06^{e}	$0.19 \pm 0.02^{a,b}$	0.79 ± 0.08^{e}
Plum	6.03 ± 0.26^{b}	281.57 ± 38.99^{a}	$10.17 \pm 0.19^{a,b}$	$0.42 \pm 0.03^{a,b}$	$0.20 \pm 0.01^{a,b}$	$0.38 \pm 0.02^{a,b}$
Dried fig	17.17 ± 1.03^{d}	1223.83 ± 89.48^{e}	17.70 ± 1.31^{f}	2.39 ± 0.24^{f}	0.35 ± 0.03^{c}	4.33 ± 0.17^{f}
Grape	23.07 ± 2.86^{e}	273.50 ± 19.82^{a}	15.36 ± 0.95^{e}	0.83 ± 0.06^{c}	$0.27 \pm 0.05^{b,c}$	$0.64 \pm 0.03^{c,d,e}$
Raspberry	2.16 ± 0.30^{a}	481.40 ± 14.50^{c}	12.73 ± 0.33^{c}	1.21 ± 0.07^{d}	0.71 ± 0.02^{d}	$0.47 \pm 0.03^{b,c}$
Apricot	$7.91 \pm 0.42^{b,c}$	$434.87 \pm 19.83^{b,c}$	9.45 ± 0.15^{a}	$0.41 \pm 0.01^{a,b}$	0.10 ± 0.01^{a}	$0.57 \pm 0.02^{c,d}$

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

fruit vinegar depends on the presence of various bioactive compounds, such as phenolic acids and flavonoids, which originate from the starting material (Haminiuk et al., 2012) and could be influenced by the processing steps involved in its production (Kelebek et al., (2016); Bakir et al., 2017; Xia et al., 2020). The six vinegars studied in the present work didnt show a positive correlation between TPC and TAC or between TFC and TAC, using a linear regression analysis (Correlation coefficient<0.50), contrary to the results obtained by Cruz et al., (2018). This situation can be explained by various factors including the effect of the numerous processing steps on the different compounds present in the raw material. The initial processing (maceration, thermal treatments, addition of nutrients), the duration of alcoholic fermentation, the acetification methods selected (slow, rapid, or submersed), and the operations eventually done in the final processing (sedimentation, filtration, pasteurization, and maturation phases) might influence the characteristics of the final products, including the chemical composition (phenolics), bioactive properties as well as sensorial properties (Cruz et al., 2018; Bakir et al., 2017). It has to be emphasized that some compounds (p. ex. phenols) can be lost during filtration steps or others (Bakir et al., 2017).

Color

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). The color properties of the vinegar samples are given in Table 3. Based on the current study, L* values of the tested samples ranged from 25.47 to 30.39, and values of a* and b* were found in the range of –0.17 to 0.48 and 2.01–3.26, respectively ($p < 0.05$). Apricot vinegar had the highest L* value. Color parameters obtained during this study demonstrated that there is a significant difference ($p < 0.05$) among the vinegars. Several studies have found a vast range of color characteristics for different types of vinegar, which also depend on raw material and processing methods [(Ozturk et al., 2015, Cruz et al. (2018), Sengun et al. (2020) and Hammouda et al. (2021)].

Mineral composition

The mineral composition differed significantly among the various samples of vinegar as summarized in Table 4, ranging between the following levels (mg/L): 2.16 – 68.67 (Na), 119.88 – 1223.83 (K), 0.54 – 17.70 (Ca), 0.29–2.39 (Mg), 0.10–5.73 (Mn) and 0.22–4.33 (Zn). The most abundant minerals are the macro elements K, Ca, Na, which are in accordance with the studies of Ozturk et al. (2015) and Hammouda et al. (2021). Dried fig vinegar was the richest in K (1223.83 mg/L), Ca (17.70 mg/mL), Mg (2.39 mg/L) and Zn (4.33 mg/L). Hammouda et al. (2021) also found high levels of K and Mg in fig vinegar. Minerals play important functions in physiologic metabolism in the structure of bones, as co-factors of enzymes, and in the regulation of osmotic pressure, among others. For example, K and Zn can generate alkaline substances, which prevent blood acidification and maintain an acid-base balance (Xia et al., 2020).

Microbiological quality

Regarding the microbiological quality of the six types of vinegar studied (fresh fig, plum, dried fig, grape, raspberry and apricot), the results from the enumeration of aerobic microorganisms at 30 ºC, filamentous fungi, yeasts, acetic acid bacteria (AAB) and lactic-acid bacteria (LAB) showed that these microbial populations were under the detection limit (1 CFU/mL). The microbial quality of vinegars depends on the technological operations to reduce the microbial load, to which the final products are subjected before bottling, such as sedimentation, clarification, filtration, and pasteurization. The presence of microorganisms, in the final product, able to use acetic acid as an energy source, could result in the spoilage of final products. Some genera of AAB, such as *Acetobacter* and *Komagataeibacter*, can transform acetic acid in CO_2 and water, which will result in a decrease of total acidity, are not desirable in vinegars (Qiu et al., 2021). In addition, the microbiological status of vinegar depends on the pH and acidity properties as well as the extrinsic factors, namely the temperature. The ethanol and lactic acid, produced mostly by yeasts and LAB during the fermentation, and the acetic acid produced during the acetification phase by AAB will certainly inhibit undesirable microorganisms, contributing to the extension of vinegar shelf-life. It should be noted that the types of vinegar studied in the present study were subjected during their final processing to filtration and pasteurization techniques in order to reduce the microbial populations and increase the shelf life of the bottled final products.

Antimicrobial capacity

In this study, the antimicrobial capacity of six different vinegar samples (fresh fig, plum, dried fig, grape, raspberry and apricot) was investigated against eight Gram + and Gram - bacterial species and seven yeast strains, using disk diffusion assay ((EUCAST 2000). Table 5 shows the antibacterial activity of the different vinegars against Gram + bacteria, namely, *Bacillus cereus* ATCC 10876, *Listeria innocua* ATCC 33090, *Staphylococcus aureus* ATCC 25923, and *Staphylococcus epidermidis* ATCC 12228, as well as the Gram - bacteria, *Cronobacter sakazakii* ATCC 29544, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella enterica* Typhimurium ATCC 14028.

The growth of *B. cereus* was most strongly inhibited by raspberry (28.67±2.31 mm) apricot (23.22±1.72 mm) and grape (22.29±6.10 mm) vinegars, with the values of the halos being not significantly different ($p > 0.05$). L. *innocua* was similarly inhibited by all the vinegars, with raspberry, apricot and grape inducing the highest inhibitions (29.75±1.89–28.13±6.66 mm) ($p < 0.05$). Regarding *S. aureus*, the highest levels of inhibition were also observed with raspberry and apricot vinegars (28.75±2.63–31.11±2.41 mm). Plum and raspberry vinegars caused the highest inhibition on the Gram + bacteria *S. epidermidis*, giving origin to halos from 29.25±1.50 mm to 29.67 ±0.58 mm, with no significant differences among the values ($p > 0.05$). The antibiotic chloramphenicol induced higher inhibitions on the Gram + bacteria than the vinegars tested (Table 5).

Of all the Gram - bacteria tested, *S.* Typhimurium was the most tolerant to the vinegars tested, with the smallest growth inhibition zones, followed by *C. sakazakii* and *E. coli*. Apricot (23.39±2.48 mm) ($p < 0.05$) was the most efficient against *S.* Typhimurium, followed by

Table 5
Antibacterial activities of vinegars from Algarve region.

Bacteria	Fresh fig	Plum	Dried fig	Grape	Raspberry	Apricot	Chloramphenicol
B. cereus	16,67 ± 2,36a	22,0 ± 3,46a,b,c	20,38±5,15a,b	22,29±6,10a,b,c	28,67 ± 2,31c,d	23,22±1,73a,b,c	31,91 ± 3,33d
L. *innocua*	25,67 ± 0,58a	26,00± 0,00a	27,75 ± 4,73a	28,13 ± 6,66a	29,75 ± 1,89a	28,83 ± 3,14a	34,81 ± 2,10b
S. aureus	23,67 ± 0,76a,b	25,0 ± 0,00a,b	21,20 ± 8,37a	21,60 ± 8,23a,b	28,75 ± 2,63a,b	31,11 ± 2,41b	30,62 ± 2,30b
S. epidermidis	19,00 ± 1,00a	29,67 ± 0,58c	18,83 ± 4,02a	25,03±3,63a,b,c	29,25 ± 1,50c	25,00±2,32a,b,c	36,33 ± 3,94d
C. sakazakii	13,00 ± 1,00a	11,33 ± 0,58a	17,60±7,23a,b	18,77 ± 8,49a,b	17,33 ± 4,18a,b	26,39 ± 1,60b	29,69 ± 1,28c
E. coli	23,33 ± 0,58c,d	16,33 ± 1,15a,b	10,75 ± 2,78a	11,50 ± 3,14a	24,83 ± 3,87c,d,e	32,00 ± 1,66f	32,25 ± 2,58f
P. aeruginosa	26,00 ± 3,61b	28,40 ± 3,21b	27,13 ± 2,87b	27,75 ± 1,71b	32,20 ± 3,03b,c,d	40,17 ± 2,72e	12,43 ± 1,90a
S. Typhimurium	11,33 ± 0,29a,b	12,67 ± 1,53a,b	11,53±3,08a,b	12,38 ± 2,56a,b	13,50 ± 1,29a,b,c	23,39 ± 2,48d	32,33 ± 2,03e

The values are given as mean ± standard deviation of triplicate determinations of inhibitory halos and are expressed in millimeters (mm); (-). Different subscripts within the same line are significantly different ($p < 0.05$).

raspberry (13.50±1.29 mm) and plum vinegar (12.67±1.53 mm). Apricot, grape and raspberry vinegars presented the highest inhibitory capacity against the growth of *C. sakazakii* with halos varying from 17.33±4.18 (raspberry) to 26.39 ± 1.60 mm (apricot) (Table 5). In regards to *E. coli*, apricot vinegar was the most inhibitory (32.00±1.66 mm) ($p < 0.05$) among the fruit vinegar experienced. *P. aeruginosa* was the most sensitive bacteria of all the bacteria tested, showing the larger growth inhibition zones in the presence of vinegars than chloramphenicol ($p < 0.05$). The greatest inhibitory vinegar against this bacterium was the apricot (40.17±2.72 mm) ($p < 0.05$) followed by raspberry (32.20±3.03 mm) and the least efficient were the fig vinegars (26.00 ±3.61 mm–27.13±2.87 mm) (Table 5).

Vinegar samples were also examined against some yeasts including *Candida albicans* ATCC 10231, *Candida albicans* ATCC 90028, *C. neoformans* YPO 186, *D. hansenii* PYCC 2968, *S. cerevisiae* PYCC 3507, *P. membranaefaciens* PYCC 2489, and *Z. bailii* PYCC 4806. The results obtained for antifungal properties are summarized in Table 4. The data on Table 6 showed that the vinegars caused levels of inhibition of yeast growth much lower than those caused in the bacteria studied (Table 5). For example, the species *C. albicans* ATCC 10231, *S. cerevisiae*, and *Z. bailli* were not inhibited by any of the vinegars, being highly tolerant to this kind of product. The species *P. membranaefaciens* was not inhibited by fresh fig and raspberry vinegars. The other vinegars induced quite small inhibition halos (6.50±0.50–7.67±0.58 mm). With regards to *C. neoformans*, grape, raspberry and apricot vinegars were the most inhibitory ($p < 0.05$) (9.67±0.298–12.17±1.44 mm). The yeast *D. hansenii* was the most sensitive to the vinegars, with the grape and raspberry vinegars inducing the greatest inhibitory actions (13.17±1.15 mm, and 15.00±1.00 mm, respectively).

The antimicrobial activity of vinegars has also been reported by several investigators using the agar diffusion method. Hammouda et al., (2021) studied the effect of several traditional Tunisian vinegars (fig, prickly pear and date) against the bacteria *E. coli, S. aureus, E. faecalis* and L. *monocytogenes*, obtaining the inhibition diameters of 10–23 mm, 15.5–24 mm, 11–17 mm and 14.5–17.5 mm, respectively. In a study by Ozturk et al., (2015) several microorganisms (*B. cereus, E. coli, E. coli* O157: H7, *Klebsiella pneumoniae*, L. *monocytogenes, P. aeruginosa, Proteus vulgaris, S.* Typhimurium, *S. aureus, Yersinia enterocolitica*) showed variable sensitivities in relation to traditionally produced types of vinegar (grape, apple, lemon, artichoke, pomegranate, hawthorn) and the growth inhibition halos were measured in a range of 6.18–23.56 mm. The species *B. cereus* was inhibited by all the vinegars studied, and it was observed that *E. coli* and *S.* Typhimurium showed lower growth inhibition halos than *B. cereus* (Ozturk et al., 2015). According to Bakir et al., (2017) vinegar samples (apple, grape, pomegranate, balsamic, blackberry, artichoke, lemon, rosehip, hawthorn, blueberry, date, blackberry, apricot and rice) showed antibacterial activity against *S. aureus* (9–13 mm), *S.* Typhimurium (9–16 mm) and *E. coli* (9–14 mm). The values of the growth inhibition zones found by these authors were, in general, lower than those observed in the present study. Similar results were also detected by Kelebek et al., (2017), who investigated apple and grape vinegars in terms of antimicrobial activity against *S. aureus* (9 - 13.33 mm), *E. coli* (9.33–14.67 mm) and *P. aeruginosa* (11.83 mm–15.50 mm).

Considering the study by Ousaaid et al., (2021), the antifungal activity of different apple cider vinegars ("red delicious", "golden delicious", "royal gala" and other varieties) was observed in two yeasts *C. albicans* and *Candida tropicalis*. The "red delicious" vinegar sample was the one that most inhibited *C. albicans* and *C. tropicalis*, with inhibition diameters between 12±0.5 mm and 11±0.7 mm, respectively, while the remaining samples did not show inhibitory effect against these yeasts. In general, in the present study, it was also observed that the yeasts tested proved to be more tolerant to vinegars than bacteria. Regarding the antifungal activity of blackberry vinegar tested on *C. albicans* (ATCC 10231) (Karaagac et al., 2016), this strain proved to be tolerant to vinegar, forming an inhibition halo of 9.6 mm, as in the present study.

In general, the Gram + bacteria studied showed growth inhibition halos higher than those measured in the Gram - bacteria, except *P. aeruginosa* ATCC 27853. In addition, the apricot and raspberry vinegars examined in this study were the ones that caused the greatest inhibition in the growth of the microorganisms investigated, especially bacteria. In some cases, grape was also associated with high bacterial inhibitory capacity. These vinegars (apricot, raspberry and grape) also had the highest acidity content (expressed as% acetic acid) and antioxidant activity values. Acetic acid is considered the major organic acid present in vinegar responsible for its bactericidal activity. This acid is a

Table 6
Antifungal (yeasts) activities of vinegars from Algarve region.

Yeasts	Fresh Fig	Plum	Dried Fig	Grape	Raspberry	Apricot	Nystatin
C. albicans (ATCC 10231)	–	–	–	–	–	–	29,20 ± 1,73
C. albicans (ATCC 90028)	8,87± 0,23a,b	8,67 ± 1,03a,b	8,50±0,63a	9,04 ± 0,46b	9,67± 0,58a,b	8,56 ± 0,68a	17,70 ± 1,51c
C. neoformans	9,83±0,29a,b,c	9,33±0,58a,b,c	8,50 ± 0,50a	9,67±0,29a,b,c	11,00±1,00a,b,c	12,17±1,44b,c	26,33 ± 1,05d
D. hansenii	11.67 ± 0.33a	12,33 ± 1,37a	11,67± 0,58a	13,17±1,15a,b	15,00 ± 1,00b	12,61 ± 1,14a,b	8,88 ± 1,13a
P. membranifaciens	–	7,67 ± 0,58b	6,50 ± 0,50a	7,17±0,29a,b	–	7,00 ± 0,00a,b	22,96 ± 3,81c
S. cerevisiae	–	–	–	–	–	–	23,21 ± 2,28
Z. bailii	–	–	–	–	–	–	21,17 ± 2,24

The values are given as mean ± standard deviation of triplicate determinations of inhibitory halos and are expressed in millimeters (mm); (-): absence of halo. Different subscripts within the same line are significantly different ($p < 0.05$).

weak organic acid and its antimicrobial potential is related to the undissociated form that is liposoluble and would cross the plasma membranes by diffusion. Once inside microbial cells, the acid will dissociate, releasing anions that accumulate intracellularly and protons that cause a reduction in the intracellular pH. The accumulation of anions and intracellular acidification are key factors in the inhibitory activity of weak organic acids and acetic acid. On the other hand, the fact that the undissociated form of weak organic acids is soluble in membrane lipids probably causes changes in its organization affecting the good performance of these membranes (Henriques et al., 1997). In addition to acetic acid and pH, the phenolic composition of vinegars derived from fruits and vegetables may contribute substantially to their antimicrobial capacity. Phenolics are secondary metabolites present in fruits and have relevant roles in the defense against microorganism infections and various other aggressions (Daglia, 2012; Haminiuk et al., 2012). The antimicrobial capacity of vinegar contributes to decreasing the number of foodborne microorganisms in vegetable foods (Sengun et al., 2020) and meat products (chicken), contributing to the extension of shelf-life (Desai et al., 2014). Microorganisms will be more or less tolerant to acetic acid and other weak organic acids, depending on their genetics, physiological and biochemical properties and ability to tolerate the accumulation of anions and acidification. Some microorganisms, such as yeast may develop mechanisms of resistance against weak organic acids including acetic acid. In fact, the species/strains of *Z. bailii, S. cerevisiae, P. membranaefaciens* and *D. hansenii* are described as highly tolerant to preservatives, such as benzoic acid (Quintas et al., 2005). The main mechanisms that have been considered to explain the resistance of some species/strains to weak organic acids are the following: a) decrease the permeability of the plasma membrane due to modification of the its characteristics, decreasing the access of the weak organic acids to the cytoplasm; b) expulsion of substances that alter the homeostatic balance (protons and anions) through membrane proteins; c) conversion of anions into less toxic compounds, through metabolization or modification of acids that accumulate intracellularly; d) capacity of yeast cells to tolerate intracellular acidification resulting from the entry of acids into cells.

The quality of vinegars results from the raw material characteristics, the procedures selected during the initial processing, the alcoholic fermentation and acetification methods as well as the final processing operations. The combination of all of these factors determine the psychochemical, bioactive and organoleptic properties of vinegars.

Conclusion

The acetification processes of non-valorized and surplus fruits resulted in vinegars with levels of acetic acid higher than 5 %, that comply with the requirements of the legislation. All the bottled final products were in excellent microbial hygienic condition. Due to the various raw materials used, all the vinegars studied displayed different properties concerning pH, acidity, color, total phenolic and flavonoid contents, minerals, antioxidant and antimicrobial properties. Potassium, calcium and sodium are the most abundant minerals in the vinegars. Dried fig vinegar stands out for its richness in macro elements and microelements (K, Ca, Mg and Zn). The grape, apricot and raspberry vinegars possessed the greatest acidity, and antioxidant activity, and were associated with the highest antibacterial activity. Among the Gram - tested, *Cronobacter sakazakii* and *Salmonella* Typhimurium were the most tolerant, while *Pseudomonas aeruginosa* was the most sensitive species, as well as some of the Gram + species tested. The yeasts, either food spoilage species or *Candida albicans* strains tested were more tolerant to vinegars than bacteria. Acetification is a strategy to valorize fruit wastes and surplus, improving environmental sustainability by minimizing the environmental impacts of these wastes and producing a food ingredient with interesting nutritional and preservation characteristics.

CRediT authorship contribution statement

Vanessa Silva: Methodology, Formal analysis, Data curation. **Gisou Mehrpour:** Methodology, Formal analysis, Data curation. **Vera Soares:** Methodology, Formal analysis, Data curation. **David Santo:** Validation, Methodology, Conceptualization. **Patrícia Nunes:** Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Célia Quintas:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

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

Data availability

The data obtained is summarized in the tables and text of the manuscript.

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