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Vinegar production from *Citrus bergamia* by-products and preservation of bioactive compounds

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Acknowledgements We express our endless gratitude to Prof Giovanni Sindona, Prof Ferdinando Delfino, and Dr Demetrio Melissari for their irreplaceable contribution concerning study conception and orchestration of the experiments. We express our sincere gratitude to Dr Nava Domenico, Dr Giuliano Delfino, and the staff of the Citrus Juices Company (Reggio Calabria) for their collaboration in the realization of the experiments.

Abstract

Processing citrus fruits results in significant amount of wastes; however, citrus by-products still contain high amounts of polyphenolic and other bioactive value-added compounds. In addition, bergamot (*Citrus bergamia*) wastes contain two compounds - brutieridin and melitidin - which exhibit statin-like properties. Recently, novel fermented products containing bioactive compounds received increasing attention because of their health-promoting functions. In this study, the bioconversion of citrus wastes in vinegars with high content in bioactive and aromatic compounds was performed, detecting a high permanence at the end of the process of the main compounds of interest, especially brutieridin and melitidin. In addition, the sensory analysis of the vinegars was performed, obtaining good performances. According to the adopted preselection procedure, 50 out of the 54 strains of acetic acid bacteria were excluded because of their low aptitude to grow in the tested conditions. The best vinegar was produced from citrus wine at pH 2.90 using the *Acetobacter aceti* strain DSM_3508^T as microbial starter. This research has demonstrated - for the first time - the possibility to produce citrus vinegar at high content of brutieridin and melitidin and other bioactive compounds using selected microbial starters.

Keywords Bioactive compounds • Brutieridin • *Citrus bergamia* • Melitidin • Vinegar

Declarations

Funding The study was not specifically funded; however, Lucia Bartella wishes to thank Italian Ministry of Education, University and Research for its grant n. AIM1899391 - 1 in the framework of the project "Azione I.2, Mobilità dei Ricercatori, PON R&I 2014–2020".

Conflict of interest/Competing interests The authors declare that they have no conflict of interest.

Ethics approval This article does not contain any studies with human or animal subjects.

Consent to participate Not applicable

Consent for publication Not applicable

Availability of data and material Not applicable

Code availability Not applicable

Introduction

Citrus contains a range of highly beneficial bioactive compounds, such as polyphenols, carotenoids, and vitamins that show antimicrobial and antioxidant properties and help in building the body's immune system. Processing citrus fruits results in significant amount of wastes; however, these citrus by-products still contain high amounts of the first-mentioned bioactive value-added compounds [1–4].

On consumption or processing, approximately 50% of the fruit remains as inedible waste, which includes peels, seeds, pulp, and segment residues; this waste still consists of substantial quantities of bioactive compounds that cause environmental pollution due to the high biological oxygen demand [5].

Bergamot (*Citrus bergamia* Risso) is cultivated almost exclusively to produce essential oils, extracted from the peel. The bergamot essence, claimed DPI since 1999 from the European Union, is widely used in the pharmaceutical industries for its antiseptic and antibacterial proprieties, in the cosmetic industries (e.g., in perfumes, body lotions, soaps, aromatherapy) for its intense fragrance and freshness, and in the food industries as aroma for the preparation of sweets, liquors and tea [6]. Bergamot peel represents about 60% of the processed fruit and is regarded as primary waste. If not processed further, it may cause environmental problems because of its high aptitude to ferment; however, bergamot peel contains very useful compounds, such as pectins and flavonoids [7]. The bergamot juice has not found a real use in the food industry and is considered a waste of the essential oil production because of its bitter taste; therefore, the disposal of bergamot juice is a serious problem - from an economical and environmental point of view - for the essential oil processing industries [8]. In addition, bergamot (*Citrus bergamia*) wastes - juice, peel, albedo, and the liquor part of the citrus waste (percolate) - contain two anticholesterolemic compounds (brutieridin and melitidin) exhibiting statin-like properties [9–12].

The bioconversion of bergamot wastes in wine and then in vinegar was recently studied; alcoholic fermentation did not modify the anticholesterolemic compounds, on the contrary, the microbial conversion of the wine into vinegar induced a significant reduction in brutieridin and melitidin [13].

Different acetic acid bacteria (AAB) species include strains that can be used to perform vinegar production. It is recognised that *Acetobacter* genus includes species (e.g. *A. aceti*, *A. malorum*, *A. pasteurianus*) which strains are able to produce about 6-8 % of acetic acid; they are mainly involved in low-medium acetic acid content vinegars making. Whereas, within *Komagataeibacter* genus some species, such as *K. europaeus*, are responsible for high acidity (15-18% acetic acid) vinegars production [14–19]. However, most of vinegar production is performed by mixed cultures of AAB, propagated by back-sloping procedure. Few examples of industrial selected starter culture

for vinegar production are available, due to physiological needs of AAB, especially in high selective environments, like vinegar [20]. Moreover, also the need of appropriate procedure to handle cultures for the successful scale-up of starters limits the application of selected starter at industrial scale [21].

Citrus vinegars exhibit a wide complexity, especially for polyphenolic and volatile profiles [22, 23]. There is an increased demand for fruit vinegars, given their reputation as a health food product [24]. There is also a growing interest towards natural compounds exhibiting statin-like activity [25].

Based on these observations, the aim of this study was to select an AAB starter able to ferment citrus wines keeping unchanged the content of brutieridin, melitidin, and other bioactive compounds.

Materials and Methods

Microorganisms

A total of 54 AAB strains, obtained from the collection of the Laboratory of Microbiology (Department of Agriculture, *Mediterranea* University of Reggio Calabria, Italy), the Unimore Microbial Culture Collection (UMCC - University of Modena and Reggio Emilia, Italy) and the German Collection of Microorganisms and Cell Cultures (DSMZ - Leibniz Institute, Braunschweig, Germany), were considered for the selection (Table 1). Most of the strains belonged to the *Acetobacter* genus (47 strains), the remaining to the genus *Komagataeibacter* (4 strains) and *Gluconacetobacter* (3 strains).

Periodical transplants of the cultures in a fresh medium GYC (glucose 10%, yeast extract 1%, calcium carbonate 2%, agar 1.5%) were performed in order to ensure growth and viability of the microbial cells used in the different fermentation trials [16].

AAB strains selection and fermentation trials

In order to check the suitability of AAB strains to conduct the acetic fermentation of citrus wine a multi-step procedure was adopted.

The procedure involved sequential trials to verify the ability of the strains to growth in specific conditions, such as: 1) citrus wine adjusted to pH 3.50 with sodium hydroxide; 2) citrus wine adjusted to pH 3.50 after the addition of citric acid (5 g/100 mL); 3) citrus wine adjusted to pH 3.50 after the addition of bergamot essential oil kindly

supplied by Citrus Juices Company (Reggio Calabria, Italy) (0.2 mL/100 mL - thoroughly mixed); 4) citrus wine adjusted to pH 3.00 after the addition of citric acid (5 g/100 mL) and bergamot essential oil (0.2 mL/100 mL - thoroughly mixed). The citrus wine was kindly produced at the Citrus Juices Company (Reggio Calabria) using filtered bergamot juice with the addition of sucrose until 14 °Brix, inoculated with the selected strain L797 of *Hanseniaspora guilliermondii*, according to our previous work [13].

Firstly, each AAB strain was pre-cultured in test tube containing 10 mL of commercial white wine (Winery Malaspina, Melito Porto Salvo - Reggio Calabria, Italy) diluted with water in a ratio of one to two and adjusted to pH 5.50; the medium was sterilised by filtration on cellulose membrane (0.22 μm) before the inoculum with the culture strain. The inoculated test tubes were incubated at 25 °C for one week. An aliquot of 2% of the pre-cultures was used to inoculate in duplicate 10 mL of each kind of the previously described modified citrus wine. The microbial growth was evaluated by measuring the optical density at 520 nm after incubation at 25 °C for one week. Each time the culture strains unable to growth were excluded from the subsequent tests. Finally, the preselected AAB, remained after the exclusion of the worst strains, were inoculated in citrus wine at pH 2.90 and in citrus wine adjusted at pH 3.50; the vinegars obtained were subjected to chemical and sensory analyses.

In order to valorise bergamot wastes, both juice and percolate were used as substrate for alcoholic fermentation. Therefore, 200 L of the following juice combination were prepared: 48% of filtered bergamot juice, 24% of water, 11% of filtered mandarin juice, 10% of sucrose, and 7% of filtered bergamot percolate (from fruit peel and albedo). This juice combination (pH 3.46 and 15.4 °Brix) was covered with liquid paraffin and inoculated at 5% (v/v) with a 48h-preculture of *H. guilliermondii* strain L797, grown in the same juice combination, previously treated at 110 °C for 5 min. The fermentation was performed at 25 °C; when the CO_2 production was totally ceased (1 month), the fermentation was considered completed and the sample was analysed, after a further 7 days of waiting.

The wine was divided in two lots - the first leaved at the original pH (2.90) and the second adjusted to pH 3.50 - and inoculated with the preselected AAB strains. This differentiation was decided in order to verify if the loss of a part of the two statin-like compounds observed in our previous work [13] depended on a pH too low. The aerobic oxidation of ethanol to acetic acid by AAB was performed at 25 °C in duplicate; after 3 months, the fermentation was considered completed and the vinegars were analysed.

Methanol and ethanol quantification

Methanol and ethanol were analysed in a Thermo Trace 1300 GC (Waltham, MA, USA) coupled with a FID detector at 280 °C and a Supelcowax 10 fused silica capillary column (0.53 mm ID, 2.00 µm film thickness, 30 m length), using the method proposed by Mei-Ling et al. [26] modified as follows. The samples were directly injected (0.1 µL) in split mode (split ratio 1:30), the injector temperature was set at 230 °C and helium was used as a gas carrier in a constant pressure of 50 kPa. The initial oven temperature was 60 °C (12 min), then it was increased to 200 °C at 10 °C/min and held at 200 °C for 10 minutes. Acetonitrile and 2-pentanol were used as internal standard respectively for ethanol and methanol determination. Different standard solutions of ethanol/acetonitrile and methanol/2-pentanol were used to calculate the relative response factor that successively was used to quantify the methanol and ethanol content.

Acetic acid quantification

AAB fermented vinegars were diluted with bi-distilled water and filtered in a 0.45 µm PTFE syringe filter Supelco (Mainz, Germany), whereupon they were injected in a HPLC (Knauer Instruments, Berlin, Germany) coupled with a Smartline pump, a DAD detector (model 2600) set at 210 nm, a 20 µL Rheodyne injection valve and with an Acclaim OA column (250 mm x 4 mm x 5 µm, Dionex Corporation, Salt Lake City, UT) at 30 °C. The flow rate was 0.6 mL/min using the following isocratic mobile phase: 100 mM Na_2SO_4 acidified to a 2.65 pH with methansulfonic acid. The quantification was obtained with the external standard method and the acetic acid content was expressed as mg/L. The method applied was suggested by the HPLC column provider.

Analysis of polyphenolic compounds

Wine and AAB fermented vinegars were diluted, filtered, and injected in the HPLC apparatus equipped with two Smartline pumps, a DAD detector (model 2600) and a 20 µL Rheodyne injection valve. The system was equipped with a C18 reversed phase Eurosphere II column (4.6 mm i.d. x 250 mm length x 3 µm particle size, Knauer Instruments, Germany). The column temperature was set at 30 °C. The flow rate was 1 mL/min. The eluents were *mobile phase A* (ultrapure water adjusted to pH 3 with formic acid), and *mobile phase B* (acetonitrile acidified to pH 3 with formic acid). The gradient used was: 95% A in isocratic for 20 min, from 95% to 60% A (20-50 min), from 60% to 5% A (50-55 min), 5% A in isocratic (55-60 min), from 5% to 95% A (60-65 min) and then an isocratic flow (5 min) to equilibrate the system before to start the new analysis. Procyanidins, flavonoids and gallic

acid were detected at 280 nm; vanillic acid was detected at 365 nm; chlorogenic acid and caffeic acid were detected at 305 nm.

Brutieridin and melitidin assay

The HMG-flavonoids brutieridin and melitidin were quantified by HPLC-UV; the wine and vinegar samples were filtered through a 0.45 μm PTFE filter, centrifuged at 2500 xg for 5 min and then injected filling a 20 μl loop. The experiments were performed using a FractionLynx System from Waters (Mildford, MA, USA) equipped with a quaternary gradient pump (Waters 2535), a UV/Visible detector (Waters 2489) working at λ=280 nm and a sample manager (Waters 2767). The HPLC separation was achieved using a Luna C_{18}(2) reverse phase column (4.6 mm i.d. x 250 mm length, 5 μm particle size; Phenomenex, Torrance, CA), using a gradient from 0.1% formic acid in water (solvent A) and methanol (solvent B). The gradient was composed by the following steps: 1) 80% A in isocratic for 7 min; 2) from 80% to 40% A (7-40 min); 3) 40% A isocratic for 5 min; 4) from 40% to 20% A (45-50 min); 5) 20% A in isocratic for 7 min; 6) from 20% to 80% A (57-62 min); the column was equilibrated by an isocratic flow (8 min) at the 80% A before starting the new analysis. The total run time was 70 min, and the flow rate was set at 1 mL/min. The concentration of brutieridin and melitidin was evaluated using an external calibration curve obtained by standard solutions of brutieridin and melitidin at 20, 40, 90, 175, 350 and 700 μg/mL. Brutieridin and melitidin were purified by literature method [9].

Total polyphenolic content

The total polyphenolic content was given as the sum of the single polyphenols.

Sensory analysis

A panel of ten human assessors (five women and five men, aged from 21 to 65), with previous experience in food and beverages sensory analysis, was recruited to preliminarily judge the eight vinegar samples. The sensory analysis was carried out on 10 mL of vinegar, placed in a disposable 50 ml plastic cup closed with a lid, at room temperature (21±2°C). The cups were coded by a random 3-digit number and the tests were performed by the same sensory panel in two different time in order to evaluate not more than four vinegar samples each time. All assessors

were provided with mineral water, inviting them to drink, after each sample tested. To start, the judges were asked to evaluate only by sniffing the samples and describe them by using 18 odour descriptions listed into the sensorial analysis form. Afterward, the assessors evaluated the taste among nine descriptions (sour, astringent, pungent, salty, bitter, spicy, umami, metallic and sweet). Finally, the data were collected and expressed as a percentage of each descriptor for each vinegar.

Statistical analysis

All the analyses were performed in duplicate; data were subjected to statistical analysis using StatGraphics Centurion XVI for Windows XP (StatPoint Technologies, Inc., USA) according to Fisher's LSD (Least Significant Difference) ($p < 0.05$). The statistical analysis was performed between all the eight vinegars derived by acetification of citrus wine. The analysis was finalized to point out if the vinegars produced were effectively different.

Ethics approval This article does not contain any studies with human or animal subjects.

Results and discussion

In our previous research [13], the strain L797 belonging to *H. guilliermondii* species was used to ferment wine samples which led to a sensorially appreciated vinegars; accordingly, the same yeast strain was used in the present study. The alcoholic fermentation did not modify the anticholesterolemic compounds, however the microbial conversion of the wines into vinegars induced significant reduction in the anticholesterolemic compounds. For this reason, we decided to set up a specific selection of AAB strains useful for citrus vinegar production and able to preserve the desired compounds. We employed different conditions to test the ability of 54 AAB strains to act as starter for fermenting citrus wine. The criteria used for selection were the ability to grow at different pH and the growth modality (formation of thin biofilm on the surface of tubes). The first medium (citrus wine adjusted to pH 3.50) - as expected - resulted the easiest to use for the most part of the AAB; the other media have proven to be progressively more restrictive for the majority of the strains. According to the preselection procedure adopted in this study, 50 out of the 54 AAB strains (Table 1) were no further considered because of the low aptitude to grow in the tested conditions. Consequently, only the following four strains were inoculated in the citrus wine: 1) *A.*

pasteurianus DL15, 2) *A. aceti* DSM_3508^T, 3) *A. malorum* DSM_14337^T, and 4) *K. hansenii* DSM_5602^T.

Table 2 highlights the analytical parameters of: a) the citrus wine, b) the four vinegars produced using the citrus wine at its original pH (2.90), and c) the four vinegars produced using the citrus wine adjusted to pH 3.50. The effectiveness of the acetic acid fermentation expressed as ethanol depletion and acetic acid increase, was optimal for all the four selected AAB strains at pH 2.90. Moreover, at the end of both fermentations (pH 2.90 and pH 3.50) the ethanol was nearly zero; this implied that part of its content was lost by evaporation, as it is often observed during static vinegar fermentations [27]. At pH 2.90 the highest acetic acid amount was produced by the strain DSM_5602^T which belong to the species *K. hansenii*. However, in static vinegar fermentations, the best performance is generally obtained by strains of the genus *Acetobacter*, which produce less amount of acetic acid respect to *Komagataeibacter* strains, but they are more suitable to develop static fermentations managed by serial refilling steps of alcoholic liquid. Indeed, strains of the species of *A. pasteurianus* and *A. aceti* are frequently isolated from vinegars produced by long static fermentations [28]. At pH 3.50 the final acetic acid produced was lower for all the strains respect to that obtained by the fermentation conducted at pH 2.90. This is commonly observed for AAB species selected for vinegar production because of their preference for low pH environments. The highest acetic acid content was produced by strains DSM_14337^T and DL15, belonging to the species *A. malorum* and *A. pasteurianus*, respectively. The total polyphenolic content of the wine significantly varied in the vinegars. The gallic acid present in the wine, in almost all the vinegars was not more detectable. On the contrary, protocatechuic acid was not detectable in the wine, but in the vinegars its content significantly varied. Protocatechuic acid has structural similarity with gallic acid; more than 500 plants contain this acid as active constituent imparting various pharmacological activity and these effects are due to their antioxidant activities, along with other possible mechanisms, such as anti-inflammatory properties and interaction with several enzymes [29]. Chlorogenic acid - which exhibits a recognised role in controlling inflammatory stress conditions [30] - decreased in all the vinegars with respect to the wine. On the contrary, the vanillic acid increased in almost all the vinegars. Caffeic acid decreased in all the vinegars. Syringic acid found in vinegar is released by the breakdown of the compound malvidin, found in red wine [31]. The syringic acid content increased considerably in all the vinegars. Epicatechin was not detectable in the wine, but in the vinegars its content significantly varied. Eriocitrin, which exhibits lipid-lowering effect [32], significantly varied in the vinegars. Neoeriocitrin, which is a recognised chemotherapeutic agent [33], significantly varied in the vinegars too. Narirutin decreased in all the vinegars. Naringin, which exhibits antioxidant activity [34], was the phenolic compound present at the highest amounts both in wines and in vinegars. Hesperidin, which is a recognised cancer chemo-protective agent [35], significantly

varied in the vinegars. Neohesperidin, which exhibits antioxidant activity [36], decreased in all the vinegars. Brutieridin and melitidin both exhibit statin-like activity. Excluding strain DL15 - which significantly reduced both brutieridin and melitidin contents at the two pH-values, the other three strains maintained almost unchanged these two statin-like compounds during the acidification process. It can be hypothesized that the presence of esterase enzymes in DL15 strain may lower the concentration of melitidin and brutieridin, exerting hydrolysis on the ester linkage of 3-hydroxy-3-methyl glutaryl moiety.

In order to evaluate the potential acceptability of the obtained vinegars on the market, a preliminary assessment of consumer preference was carried out on the vinegar samples. The results of the sensory analysis of the eight vinegars, performed using nine taste descriptors and 18 odour descriptors, were reported in Table 3. The data, expressed as a percentage of each descriptor for each vinegar, allowed to clearly observe the frequency of the descriptors themselves. In particular, all the samples exhibited a sour taste as well as a high astringent taste (range of percentage 80-100). Moreover, the most frequently chosen descriptors for vinegar taste were astringent, pungent, salty, bitter, spicy and umami. Generally, this is in accordance with the taste of vinegar which is considered to be dominated by sour, followed by sweet and umami taste, slight salty and bitterness [24,37]. Regarding aroma, the most frequently chosen descriptors for the odour of the vinegar samples were floral, medicinal, apple, vinegar, sweet, oxidized, roasty and alcoholic. Commonly, floral and apple are appreciated aroma attributes in the sensory evaluation of citrus vinegar, while medicinal, oxidized or rancid are considered the worst ones. Accordingly, the samples exhibiting the highest percentage of the worst descriptors were excluded, while the vinegar sample with the highest percentage of appreciated aroma was found to be the one produced from the citrus wine at its original pH and using *A. aceti* strain DSM_3508^{T} as a starter culture.

The acetic acid production by the strains used showed that the adjusting pH at a value of 3.50 is not useful to protect bioactive compounds, but the acidity level naturally reached after alcoholic fermentation gave the best results. The slight increase of pH of vinegars respect the original wine could be due to a consumption of citric acid and the corresponding production of acetic acid. The first acid shows a pKa of the first dissociated proton of 3.13, the second, a weaker acid, shows a pKa of 4.76. Furthermore, the increase of pH of vinegars obtained from wines with adjusted pH at 3.50 is obviously due to this operation. Commercial strains specific for vinegar (DSMZ) maintained the content of melitidin and brutieridin similar of those revealed in the starting citrus wine. So, the importance of a correct and precise strain selection is validated. The strain DL15, also isolated from a vinegar, gave similar results to others respect for melitidin and brutieridin that could be loss for the action of microorganism or adsorbed on it.

Conclusion

Annually, the citrus waste accumulated by processing industries is estimated to be over 60 million tons worldwide [9]. However, the citrus waste can be utilized for obtaining valuable bioactive compounds employing extraction and purification techniques [5]. In recent years, researchers across the world have been focusing on developing various processing methods for maximum exploitation as well as utilization of various waste products of citrus fruits. Waste fermentation to ethanol using either *Saccharomyces cerevisiae* or recombinant strains of *Escherichia coli* or a combination of both was reported [38-39]. Citrus wastes are best utilized to obtain fibres and food ingredients, e.g., pectins and mucilages [40-41]. The solid and highly concentrated liquid citrus wastes are transformed into citrus molasses, feed yeast, lactic acid, industrial alcohol, vinegar, etc. [42]. In addition to this, recently novel fermented products containing bioactive compounds received increasing attention because of their health-promoting functions. This is also the case of vinegar sector, which is moving through the introduction on the market of new products that differ from conventional one for the raw material, the degree of acetic acid, and the content of bioactive compounds. With the present research, the bioconversion of citrus wastes in vinegars with high content in bioactive and aromatic compounds was performed, detecting a high permanence at the end of the process of the main compounds of interest, especially brutieridin and melitidin. In addition, the outputs obtained from the consumer's acceptance test highlighted the potential introduction of this vinegar on the market. Based on the results obtained, it is evident that the pH does not influence the degradation of the bioactive components, whereas is instead strain-dependent. It is important to highlight the valorisation of the bergamot pulp, which confers to the citrus vinegar peculiar chemical and nutraceutical characteristics. The outputs of this study highlight the possibility to use selected starter culture of AAB which exhibit both the ability to produce suitable amount of acetic acid (which is a required attribute) while maintaining the brutieridin and melitidin. Thus, a double selected starter of AAB composed of *A. aceti* and *A. pasteurianus* strains could be the rational option to produce vinegar from citrus by-products through static fermentation regime. In this study, it was also demonstrated, for the first time, the possibility to produce citrus vinegar using selected starter cultures of AAB, which not negatively affect the content of brutieridin and melitidin and other bioactive compounds. The selected strain belongs to the *A. aceti* species, which strains are generally involved in the production of vinegars at low-medium acetic acid.

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Table 1 List of the 54 strains of acetic acid bacteria studied.

Strain code*	**Species**	**Source**
AB021=UMCC 1743	*Acetobacter malorum*	vinegar
B67	*Acetobacter spp.*	grape must
B68	*Acetobacter spp.*	grape must
B95	*Acetobacter spp.*	acescent wine
B96	*Acetobacter spp.*	acescent wine
B97	*Acetobacter spp.*	acescent wine
B98	*Acetobacter spp.*	acescent wine
B99	*Acetobacter spp.*	acescent wine
B100	*Acetobacter spp.*	acescent wine
B101	*Acetobacter spp.*	acescent wine
B103	*Acetobacter spp.*	acescent wine
B104	*Acetobacter spp.*	acescent wine
B105	*Acetobacter spp.*	acescent wine
B106	*Acetobacter spp.*	acescent wine
B107	*Acetobacter spp.*	acescent wine
B108	*Acetobacter spp.*	acescent wine
B109	*Acetobacter spp.*	acescent wine
B110	*Acetobacter spp.*	acescent wine
B111	*Acetobacter spp.*	acescent wine
B112	*Acetobacter spp.*	acescent wine
B113	*Acetobacter spp.*	acescent wine
B114	*Acetobacter spp.*	acescent wine
B115	*Acetobacter spp.*	acescent wine
B116	*Acetobacter spp.*	acescent wine
B191	*Acetobacter spp.*	acescent wine
B192	*Acetobacter spp.*	acescent wine
B194	*Acetobacter spp.*	acescent wine
B195	*Acetobacter spp.*	acescent wine
B196	*Acetobacter spp.*	acescent wine
B197	*Acetobacter spp.*	acescent wine
B198	*Acetobacter spp.*	acescent wine
B258	*Acetobacter spp.*	acescent wine
B264	*Acetobacter spp.*	acescent wine
B265	*Acetobacter spp.*	acescent wine
B296	*Acetobacter spp.*	acescent wine
B547	*Acetobacter spp.*	acescent wine
B548	*Acetobacter spp.*	acescent wine
B549	*Acetobacter spp.*	acescent wine
B550	*Acetobacter spp.*	acescent wine
DL13=UMCC 1786	*Acetobacter pasteurianus*	cereal vinegar
DL15=UMCC 1787	*Acetobacter pasteurianus*	cereal vinegar
DL21A=UMCC 1788	*Acetobacter pasteurianus*	cereal vinegar
DSM_2325	*Komagataeibacter xylinus*	-
DSM_3508^{T}	*Acetobacter aceti*	vinegar
DSM_3509^{T}	*Acetobacter pasteurianus*	beer
DSM_5601^{T}	*Gluconacetobacter diazotrophicus*	sugarcane roots
DSM_5602^{T}	*Komagataeibacter hansenii*	vinegar
DSM_5603^{T}	*Gluconacetobacter liquefaciens*	dried fruit
DSM_6160^{T}	*Komagataeibacter europaeus*	vinegar
DSM_6513^{T}	*Komagataeibacter xylinus*	mountains ash berries
DSM_12717^{T}	*Gluconacetobacter sacchari*	mealy bug from sugar cane
DSM_14337^{T}	*Acetobacter malorum*	rotting apple
L7=UMCC 1735	*Acetobacter pasteurianus*	vinegar
ZJ25B=UMCC 1800	*Acetobacter pasteurianus*	cereal vinegar
* Microbial Culture Collections: UMCC = Unimore Microbial Culture Collection; DSM = Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures.		

Table 2 Analytical parameters of the citrus wine and of the vinegars produced using it at its original pH (2.90) and adjusted to pH 3.50

	Wine	Vinegars from wine at pH 2.90				Vinegars from wine at pH 3.50			
Microbial starter	**L797**	**DL15**	**DSM_3508^T**	**DSM_14337^T**	**DSM_5602^T**	**DL15**	**DSM_3508^T**	**DSM_14337^T**	**DSM_5602^T**
pH	2.90	2.97	2.98	2.98	2.97	3.42	3.46	3.48	3.49
Ethanol (vol.%)	7.60±0.07	0.15±0.02^{b}*	0.15±0.02^{b}	0.16±0.01^{b}	0.14±0.02^{b}	0.14±0.01^{b}	0.15±0.01^{b}	0.21±0.00^{a}	0.15±0.01^{b}
Methanol (vol.%)		0.02±0.00^{b}	0.02±0.00^{b}	0.01±0.00^{b}	0.01±0.00^{b}	0.01±0.00^{b}	0.02±0.00^{b}	0.03±0.00^{a}	0.01±0.00^{b}
Acetic acid (g/L)		45.067±0.008^{a}	45.402±3.435^{a}	43.410±0.680^{ab}	45.548±2.201^{a}	40.789±0.141^{b}	35.463±0.147^{c}	40.868±1.384^{b}	29.687±0.839^{d}
Total polyphenols (mg/L)	791±10	698±1^{d}	762±3^{c}	762±4^{c}	693±4^{d}	618±5^{e}	766±6^{bc}	777±6^{b}	826±6^{a}
Gallic acid (mg/L)	4.12±0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.11±0.05	<0.01
Protocatechuic acid (mg/L)	<0.01	3.41±0.03^{b}	2.82±0.03^{c}	2.34±0.36^{d}	1.80±0.02^{e}	2.03±0.08^{de}	2.85±0.18^{c}	3.31±0.30^{b}	3.81±0.02^{a}
Chlorogenic acid (mg/L)	6.91±0.48	4.78±0.02^{cd}	6.06±1.23^{ab}	4.20±0.12^{d}	4.53±0.80^{d}	4.83±0.13^{cd}	6.25±0.07^{ab}	6.82±0.01^{a}	5.60±0.12^{bc}
Vanillic acid (mg/L)	1.60±0.38	3.20±0.03^{a}	2.37±0.23^{bc}	1.75±0.33^{cd}	1.88±0.58^{cd}	2.79±0.61^{ab}	1.49±0.04^{d}	2.40±0.09^{bc}	2.05±0.50^{bcd}
Caffeic acid (mg/L)	6.96±0.33	1.89±0.02^{c}	1.63±0.24^{d}	1.17±0.12^{e}	1.79±0.09^{cd}	1.85±0.01^{cd}	2.31±0.03^{b}	2.26±0.15^{b}	2.70±0.05^{a}
Syringic acid (mg/L)	1.81±0.19	14.09±0.06^{a}	12.57±1.31^{ab}	11.42±1.21^{b}	12.81±0.19^{ab}	12.75±0.24^{ab}	14.21±0.35^{a}	14.03±1.58^{a}	13.32±0.10^{a}
Epicatechin (mg/L)	<0.01	15.81±0.09^{b}	15.97±0.20^{b}	11.52±0.32^{d}	15.61±1.46^{bc}	18.84±0.41^{a}	13.64±0.72^{c}	15.92±0.75^{b}	18.52±2.29^{a}
Eriocitrin (mg/L)	15.82±0.16	16.96±0.19^{a}	16.37±1.67^{ab}	12.92±0.25^{c}	15.40±0.12^{b}	15.11±0.04^{b}	17.27±0.35^{a}	15.20±0.48^{b}	16.35±1.05^{ab}
Neoeriocitrin (mg/L)	228.40±1.32	213.87±2.14^{b}	211.85±11.06^{b}	222.59±0.50^{ab}	181.95±1.20^{e}	191.36±6.66^{de}	204.12±8.65^{cd}	207.61±1.43^{bc}	233.38±14.83^{a}
Narirutin (mg/L)	14.01±1.51	8.25±0.07^{e}	7.79±0.07^{f}	9.62±0.08^{b}	8.05±0.11^{e}	9.34±0.12^{c}	8.22±0.04^{e}	9.01±0.03^{d}	10.41±0.08^{a}
Naringin (mg/L)	230.14±5.01	241.13±2.40^{ab}	229.11±6.85^{b}	229.30±9.37^{b}	202.88±4.31^{c}	197.65±0.97^{c}	237.47±10.04^{ab}	241.54±6.82^{ab}	254.22±17.01^{a}
Hesperidin (mg/L)	10.48±1.05	13.05±0.04^{a}	10.65±1.32^{b}	8.21±0.56^{c}	9.77±0.03^{bc}	7.81±1.08^{c}	9.18±0.75^{bc}	9.78±1.75^{bc}	10.58±1.13^{b}
Neohesperidin (mg/L)	129.27±0.80	115.97±0.47^{ab}	110.21±2.65^{abc}	114.89±0.93^{ab}	101.73±0.94^{c}	107.79±9.12^{abc}	113.92±3.65^{ab}	116.61±3.87^{ab}	118.46±7.13^{a}
Brutieridin (mg/L)	75.46±0.96	26.15±0.89^{b}	73.78±2.36^{a}	72.66±2.65^{a}	74.02±0.96^{a}	25.47±0.56^{b}	74.50±2.21^{a}	73.17±2.17^{a}	74.71±1.48^{a}
Melitidin (mg/L)	65.97±0.45	19.71±0.23^{b}	60.63±1.55^{a}	59.80±1.62^{a}	60.88±1.97^{a}	19.94±0.87^{b}	60.65±1.12^{a}	59.67±1.14^{a}	61.52±1.30^{a}

*Values followed by different small letters in the same row (wine excluded) are significantly different ($p<0.05$)

Table 3 Sensory analysis of the eight vinegars; data are expressed as a percentage of each descriptor for each vinegar

		Vinegars from citrus wine at its original pH (2.90)				Vinegars from citrus wine adjusted to pH 3.50			
	Microbial starter	**DL15**	**DSM_3508^T**	**DSM_14337^T**	**DSM_5602^T**	**DL15**	**DSM_3508^T**	**DSM_14337^T**	**DSM_5602^T**
TASTE	Sour	100	100	100	100	100	100	100	100
	Astringent	100	100	100	90	80	100	100	80
	Pungent	100	100	100	90	80	80	90	100
	Salty	90	50	70	90	80	90	70	80
	Bitter	40	70	80	80	50	70	80	100
	Spicy	30	30	50	70	40	50	60	40
	Umami	20	60	50	60	40	50	40	50
	Metallic	40	30	50	40	40	40	40	40
	Sweet	20	30	40	30	20	30	50	20
AROMA	Floral	40	60	40	40	30	30	30	40
	Medicinal	10	10	20	50	20	40	30	30
	Apple	20	40	40	20	0	50	20	10
	Vinegar	30	30	10	20	10	30	30	30
	Sweet	20	40	10	10	0	20	20	20
	Oxidized	10	10	30	0	20	10	30	20
	Roasty	20	10	20	30	20	10	0	20
	Alcoholic	0	20	20	0	20	10	40	10
	Citrus	10	10	20	0	20	10	0	10
	Mushroom	10	0	20	10	20	10	0	10
	Fruity	10	0	10	0	10	20	10	10
	Herbaceous	20	0	0	10	20	10	10	0
	Caramel	20	0	0	10	10	0	10	0
	Woody	20	0	10	0	0	0	0	10
	Cheese	10	0	0	10	0	0	0	10
	Leather	0	10	0	0	0	10	0	10
	Rancid	0	0	0	0	0	10	0	10
	Liquorice	0	10	0	0	0	0	0	0