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Vinegar production to valorise *Citrus bergamia* by-products

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Abstract

In the bergamot (*Citrus bergamia*) processing cycle, peel and juice are the main by-products. Considering their high content in bioactive and aromatic compounds, the aim of this study was to valorise them in the vinegar industry. The proposal constitutes a model system for the global citrus industry, to improve the commercial value of the citrus wastes. The bioconversion of four juice combinations (based on bergamot fruit peel or juice) in eight wines and, after random choice, in four vinegars was tested. The chemical composition of wines and vinegars was determined, detecting a high permanence of a majority of the compounds of interest. The sensory analysis of the four vinegars before and after an oxidation treatment was performed obtaining good performances.

Keywords By-products · *Citrus bergamia* · Model system · Vinegars

Introduction

The genus *Citrus* is grown widely all over the world; processing citrus fruits results in a significant amount of waste (peels, seeds, and pulps), which accounts for 50% of the fruit [1]. Citrus by-products are a valuable source of carotenoids, dietary fibres, soluble sugars, cellulose, hemicellulose, pectin, polyphenols, ascorbic acid, methane, and essential oils [2–7]. However, in Italy and in many other Mediterranean countries, citrus fruit peels are frequently a wasted resource; the surplus is not directly usable at the wet state and its drying process is too expensive. Therefore, the residual part is eliminated by waste disposal sites, by incineration or by other costly breaking-down systems. Due to the low pH and to the presence of easily fermentable sugars and organic acids in the citrus by-products, it is feasible a low-cost two-step fermentation process to produce citrus wine and vinegar. At present, in the world global market it is possible to get many flavoured vinegars based on citrus fruits; in addition, citrus by-products may be an important source of bioactive constituents, with health-promoting and nutritive properties [8, 9]. Bergamot (*Citrus bergamia*) juice has high content in phenolic and other antioxidant, and aromatic compounds [10–12]; moreover, analogously to bergamot albedo, it has two anticholesterolemic compounds (brutieridin and melitidin) exhibiting statin-like properties [13–16]. Generally, bergamot juice is not directly drunk, due to its intense bitter taste. Although it is possible to obtain a flavoured wine from bergamot juice, it is not produced for direct consumption, due to the risk related to a relatively high methanol content, produced from citrus pectin during winemaking. The aim of this study was to valorise in the vinegar industry the bergamot by-products. The proposal constitutes also a model system for the global citrus industry. Therefore, bergamot fruit peel, albedo, percolate, and juice exploit their high content in bioactive and aromatic compounds. The bioconversion of bergamot juice in wine and then in vinegar was preliminarily studied [17, 18]. The present contribution aimed to evaluate if the double fermentative process (alcoholic fermentation and acetic oxidation) maintained unchanged the bioactive compound content of the citrus waste giving a product with positive sensory properties stable in the time. In addition, our aim was to give a complete description of the composition in bioactive and aromatic compounds of the intermediate and the final products, highlighting their complexity.

Materials and methods

Materials

Eight litres of the following juice combinations (based on bergamot fruit peel or juice) have been prepared: (1) filtered bergamot juice with the addition of saccharose until 14 °Brix (BS); (2) filtered bergamot juice and filtered mandarin juice (1:1) with the addition of saccharose until 14 °Brix (BMS); (3) filtered bergamot percolate (from fruit peel and albedo) with the addition of saccharose until 14 °Brix (PS); (4) filtered bergamot percolate (from fruit peel and albedo) and filtered mandarin juice (1:1) with the addition of saccharose until 14 °Brix (PMS) (Table 1). Each juice combination was divided into two lots of 4 litres, covered with 100 mL of liquid paraffin, and inoculated with 5% (v/v) concentration of 48-h yeast precultures. Therefore, the two specifically selected yeast strains Sc734 of *Saccharomyces cerevisiae* and L797 of *Hanseniaspora guilliermondii*, grown in the same juice combination previously treated at 110 °C for 5′, were inoculated. The fermentations were performed at 25 °C; when the CO_2 production ceased (25–30 days), the fermentations were considered completed and the samples were refrigerated for 48 h at 4 °C, decanted, and analysed. Four out the eight wines, randomly chosen, were inoculated with a blend of four different strains of specifically selected *Acetobacter* strains. The fermentations were performed at 25 °C; after 3 months, the vinegars were analysed.

ABTS assay
It is the determination of the radical scavenging activity. The method proposed by Re et al. [19] was here applied and modified as follows. A 0.010–0.025 mL aliquot of the untreated sample was placed in a glass vial and a 7 mM ABTS ethanol solution was added to a final volume of 2.5 mL. The variability of the sample was due to the different antioxidant potential of each sample. The mixture was vigorously shaken in the dark (6 min). At this point, the absorbance was read immediately (*t*0) and after 6 min (*t*6) at 734 nm in an Agilent spectrophotometer, model 8453 (Santa Clara, CA, USA). The following formula, Percentage inhibition = $[(t0 - t6)/t0] \times 100$, was used to express the results.

DPPH assay
It is the determination of the radical scavenging activity based on the reduction of the stable free radical 2.2-diphenyl-1-picrylhydrazyl (DPPH). The method proposed by Brand-Williams et al. [20] was modified as follows. In a glass vial were added 0.025 mL of sample and 2.475 mL of a 10−4 M DPPH· solution, previously prepared with methanol. The absorbance of the mixture was immediately read at 515 nm (*t*0) and after 5 min of incubation (*t*5). The following formula, Percentage inhibition = $[(t0 - t5)/t0] \times 100$, was used to express the results.

Organic acid analysis
Organic acid analysis was conducted on a Knauer chromatograph equipped with a UV detector set at 210 nm, and a 20-µL Rheodyne injection valve. The system was coupled with an Acclaim OA5 column (4 mm i.d. × 250 mm length × 5 µm particle size). Mobile phase was 100 mM Na2SO4 acidified to 2.65 pH with methane sulfonic acid CH3SO3H at 30 °C; flow rate was 0.6 mL/min. Before analysis, the untreated sample was diluted with deionised water and filtered in a 0.45-µm PTFE syringe filter Supelco.

Phenol analysis
Phenolic compounds analysis was conducted on a Knauer 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. × 250 mm length × 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 with formic acid to pH 3). 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. Also for this analysis, the untreated sample was diluted with deionised water and filtered in a 0.45-µm PTFE syringe filter Supelco. Procyanidins, flavonoids and gallic acid were detected at 280 nm; vanillic acid, rutin and quercetin were detected at 365 nm; neochlorogenic acid, chlorogenic acid and caffeic acid were detected at 305 nm.

Volatile organic compound analysis
A 10 mL aliquot of each wine and vinegar sample was placed in a 20 mL glass vial with septum cup and a magnetic stirrer was placed inside. The sample was placed in a thermostatic bath at 40 °C in stirring (500 rpm). A solidphase microextraction (SPME) fibre coated with polydimethylsiloxane (PDMS, 100 µm) from Supelco (Bellefonte, PA, USA) was used to absorb the VOCs with the procedure described by Cirlini et al. [21], modified as follows. Absorption was halted after 20 min and the fibre was thermally desorbed for 4 min in a gas chromatographer in splitless mode (injector temperature 230 °C). All SPME operations were conducted manually. A GC Thermo Trace 1310 apparatus (Waltham, MA, USA) equipped with Single Quadrupole Mass Spectrometer ISQ LT system and a TG-5MS 5% phenyl fused silica capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness, Thermo Scientific, Whaltam, MA, USA), was used for the GC–MS analysis. The oven temperature was initially programmed at 50 °C (3 min), then from 50 °C to 200 °C at 5°C/min, and maintained isothermally at 200 °C (15 min). Mass spectrometry transfer line and ion source temperatures were 250 and 260 °C, respectively. The mass range was from 40 to 500 atomic mass unit. Gas carrier (He) was set at 1 mL/min flow rate. Identification of the VOCs was based on the comparison of spectra consistent with NIST/EPA/NIH Mass spectral library Version 2.0.

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. [22] 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 He 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 min. 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.

Brutieridin and melitidin assays
The HMG-flavonoids brutieridin and melitidin were quantified by HPLC–UV, directly injecting the wine and vinegar

samples after a filtration (0.45-μm PTFE) and a centrifugation step (2500*g*, 5 min). The analyses 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) and a sample manager (Waters 2767). The chromatographic separation was achieved using a Luna C18(2) reverse phase column (4.6 mm i.d. × 250 mm length, 5 μm particle size; Phenomenex, Torrance, CA), under gradient conditions using as eluents 0.1% formic acid in water (A) and methanol (B). The gradient steps were 80% A in isocratic for 7 min, from 80 to 40% A (7–40 min), 40% A isocratic for 5 min, from 40 to 20% A (45–50 min), 20% A in isocratic for 7 min, from 20 to 80% A (57–62 min) and then an isocratic flow (8 min) to equilibrate the system before starting the new analysis. The total run time was 70 min, while the flow rate was set at 1 mL/min and the UV detector was set at 280 nm. The injection volume was 20 μL. The concentration of brutieridin and melitidin was evaluated using an external calibration curve gained by standard solutions of brutieridin and melitidin at 20, 40, 90, 175, 350 and 700 μg/mL. The pure standards brutieridin and melitidin were obtained as reported elsewhere [13].

Sensory analysis

Panels of human assessors, specifically trained, have judged the four vinegars before and after an oxidation treatment, performed leaving the samples into unplugged bottles at room temperature for 40 days.

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 wines—derived by fermentation of four different juice combinations—and between all the four vinegars—derived by acetification of wines obtained from three different juice combinations. The analysis was finalized to point out if the wines and vinegars produced were effectively different.

Results and discussion

Table 2 highlights the main analytical parameters of the eight wines. Table 3 highlights the same parameters of the four vinegars. The total acidity exhibits a very wide variability, in a range of 43.50 g/L among the eight wines and of 32.25 g/L among the four vinegars. It depends on all the process parameters, above all on the juice combination; however, it is interesting to note that this value is significantly higher in three out the four wines produced by *Hanseniaspora* compared to the corresponding wine produced by *Saccharomyces*. The vinegar showing the highest total acidity is PMS-*Hanseniaspora*. In addition, the acetic acid content exhibits an extreme variability, in the range of 13.116 g/L among the eight wines and even 103.76 g/L among the four vinegars; the vinegar showing the highest acetic acid content is PMS-*Hanseniaspora*. It is interesting to note that in the wines produced using the combinations containing mandarin juice (BMS and PMS) *Hanseniaspora* produces less acetic acid compared to *Saccharomyces*; on the contrary, in the other combinations, *Hanseniaspora* produces more acetic acid compared to *Saccharomyces*. In most of the wines, the ethanol produced overcomes the content foreseeable based on the °Brix, that is 7.40 vol. %. Probably the pectin hydrolysis has contributed to increase the ethanol content of the wines, which varies in the range of 2.92 vol. % among the eight wines. Unusually, the ethanol content of three out the four wines produced by *Hanseniaspora* is higher compared to the corresponding wines produced by *Saccharomyces*. In none of the four vinegars, the ethanol is detectable. The methanol content exhibits good levels and very low variability, in a range of just 0.05 vol. % among the eight wines and of 0.01 vol. % among the four vinegars. Table 4 highlights the analytical parameters related to the bioactive compounds and to the antioxidant activity of the eight wines. Table 5 highlights the same parameters of the four vinegars. The total phenolic content of the wines is affected by the initial content of the four juice combinations used; however, it is interesting to note that this value is significantly higher in the wine produced from PS by *Saccharomyces* compared to the corresponding wine produced by *Hanseniaspora*. On the contrary, the total phenolic content of the wine PMS-*Saccharomyces* is significantly lower compared to the corresponding wine PMS-*Hanseniaspora*. In the four vinegars, the total phenolic content is comparable to the corresponding wine; consequently, the vinegar showing the highest content is BS-*Hanseniaspora*. Chlorogenic acid, which exhibits a recognised role in controlling inflammatory stress conditions [23], is higher in three out the four wines produced by *Saccharomyces* compared to the corresponding wines produced by *Hanseniaspora*. This compound increases after acidification in three out the four vinegars, exhibiting the highest content in BS-*Hanseniaspora*. Procyanidin A, which exhibits a recognised role in vascular health [24, 25], is present only in three out the eight wines, all produced from juice combinations containing mandarin juice; consequently, only the two vinegars produced from PMS show this compound. Rutin, which exhibits a recognised antioxidant activity [26], is significantly higher in three out the four wines produced by *Hanseniaspora* compared to the corresponding wines produced by *Saccharomyces*. In the four vinegars, this compound is comparable to the corresponding wine; consequently, the vinegar showing the highest content is BS-*Hanseniaspora*. Eriocitrin, which exhibits lipid-lowering effect [27], is significantly higher in the four wines produced by *Saccharomyces* compared to the corresponding wines produced by *Hanseniaspora*. In the four vinegars, this compound is comparable to the corresponding wine; consequently, the vinegar showing the highest content is BS-*Hanseniaspora*. Neoeriocitrin, which is a recognised chemotherapeutic agent [28], is significantly higher in three out the four wines produced by *Hanseniaspora* compared to the corresponding wines produced by

Saccharomyces. In the four vinegars, this compound is comparable to the corresponding wine; consequently, the vinegar showing the highest content is BS-*Hanseniaspora*. Naringin, which exhibits antioxidant activity [29], is the phenolic compound present at the highest amounts both in wines and in vinegars; the vinegar showing the highest content is BS-*Hanseniaspora*. Hesperidin, a recognised cancer chemoprotective agent [30], is significantly higher in the four wines produced by *Saccharomyces* compared to the corresponding wines produced by *Hanseniaspora*. In the four vinegars, this compound is comparable to the corresponding wine; consequently, the vinegar showing the highest content is BS-*Hanseniaspora*. Neohesperidin, which exhibits antioxidant activity [31], is higher in three out the four wines produced by *Hanseniaspora* compared to the corresponding wines produced by *Saccharomyces*. In the four vinegars, this compound is comparable to the corresponding wine; consequently, the vinegar showing the highest content is BS-*Hanseniaspora*. Most of these values are in higher quantity with respect to data found in South Italian fresh blood orange juice (*Citrus sinensis* (L.) Osbeck cv. Moro), i.e. 1.01 mg/L (rutin), 0.40 mg/L (neoeriocitrin), 2.81 mg/L (naringin), and 0.40 mg/L (neohesperidin) [32]. The antioxidant potential of wines and vinegars is a parameter commonly studied in many food matrices and plant extracts such as strawberry tree fruits [33], coffees [34], *Opuntia ficus-indica* cladodes and fruits [35], quinces [36], and edible vegetable oils [37–39]. The antioxidant activity measured by ABTS assay is higher for the wines produced using PMS combination while it is lower for the wines produced using PS combination. The antioxidant activity measured by DPPH assay is higher for the wines produced using BS combination while is lower again for the wines produced using PS combination. In the vinegars, each vinegar is significantly different compared to all the others for the antioxidant activity, measured both by ABTS and by DPPH assays. Brutieridin and melitidin both exhibit statin-like activity [13–16]. Comparing the wines produced using the same combination, significant differences for the BS, PMS, and PS wines are observed while no significant differences are observed in the BMS wines. In general, the maximum content of molecules with statin-like activity is produced using BS while the lowest values are reported using PMS; the vinegar showing the highest content is BS-*Hanseniaspora*. Table 6 highlights volatile compound characterisation of the eight wines. Table 7 highlights the same parameters of the four vinegars. Limonene, which exhibits antioxidant activity [40], is significantly higher in three out the four wines produced by *Hanseniaspora* compared to the corresponding wines produced by *Saccharomyces*; the vinegar showing the highest content is PMS-*Hanseniaspora*. Linalool, a recognised anti-oxidative stress agent [41], is significantly higher in the three out the four wines produced by *Saccharomyces* compared to the corresponding wines produced by *Hanseniaspora*. In the four vinegars, the content in this compound is notably reduced, probably due to the activity of the acetic acid bacteria. Evidently, the high acidity—and the low pH—has a significant influence on the alteration of some terpenes—such as linalool—which decreases considerably from wine to vinegar. Anyway, the vinegar showing the highest content is PMS-*Saccharomyces*. α-Terpineol, a recognised anti-inflammatory compound [42], is significantly higher in three out the four wines produced by *Hanseniaspora* compared to the corresponding wines produced by *Saccharomyces*; the vinegar showing the highest content is BS-*Hanseniaspora*. The sensory analysis showed that all the four vinegars after the oxidation treatment became evidently darker. Now the more frequently chosen descriptors are listed for each vinegar, before and after the oxidation treatment.

- BS-*Hanseniaspora* before: citrus taste (64%) and flower (27%).
- BS-*Hanseniaspora* after: citrus taste (50%), detergent (22%) and oxidized apple (22%).
- PS-*Hanseniaspora* before: white vinegar (91%) and citrus taste (27%).
- PS-*Hanseniaspora* after: white vinegar (72%), citrus taste (22%), and herbaceous (22%).
- PMS-*Hanseniaspora* before: citrus taste (45%), flower (27%).
- PMS-*Hanseniaspora* after: fruity (56%), flower (39%), and citrus taste (33%).
- PMS-*Saccharomyces* before: vinegar (36%), flower (27%), rancid (18%), and apple (18%).
- PMS-*Saccharomyces* after: oxidized apple (28%), citrus taste (17%), flower (17%), and cheese (17%).

The PMS-*Hanseniaspora* is the vinegar resulting the best at the sensory analysis before and after the oxidation treatment.

Conclusion

With the present research, the bioconversion of four juice combinations—based on bergamot fruit peel or juice—in eight wines and, after random choice, in four vinegars has been tested. The composition in bioactive and aromatic compounds of the intermediate and the final products has been determined, detecting a high permanence—at the end of the two processes—of a majority of the compounds of interest. In addition, the sensory analysis of the four vinegars before and after an oxidation treatment has been performed obtaining good performances. Different vinegars have been obtained from different combinations of bergamot by-products. The vinegars are potentially acceptable by the consumer and have a different stability against the oxidation. They contain interesting bioactive compounds. Finally yet importantly, the vinegars with low acidity content may be used for alternative dressing or beverages. The results here presented throw light on the possible use of flavoured methanol-free bergamot wine and/or bergamot vinegar in the control of blood cholesterol level. The present work represents a model system for the citrus industry and may help other researchers to create new knowledge in the sector applying these observations in analogous backgrounds.

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Compliance with ethical standards

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

Compliance with ethical requirements This article does not contain any studies with human or animal subjects.

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Table 1 Configuration of the eight juice combinations

	BS		BMS		PS		PMS	
	H	*S*	*H*	*S*	*H*	*S*	*H*	*S*
Filtered bergamot juice (B)	x	x	x	x				
Filtered mandarin juice (M)			x	x			x	x
Filtered bergamot percolate (P)					x	x	x	x
Saccharose until 14 °Brix (S)	x	x	x	x	x	x	x	x

Table 2 Main analytical parameters of the eight wines

	BS		BMS		PS		PMS	
	H	*S*	*H*	*S*	*H*	*S*	*H*	*S*
pH	2.87a	2.92b	3.13c	3.15c	3.25D	3.40e	4.22f	4.20f
Total acidity (g/L of tartaric acid)	51.38g	47.63f	31.13e	28.13d	13.88C	7.88a	8.63b	8.63b
Acetic acid (g/L)	0.792b	0.425a	0.515a	0.813b	13.541F	2.837c	8.192d	8.562e
Ethanol (vol%)	9.23bc	8.72abc	8.05a	7.77a	8.65ab	9.69c	10.69d	7.98a
Methanol (vol%)	0.10c	0.10c	0.08a	0.08a	0.10bc	0.13d	0.10bc	0.09b

Identical letters in row indicate no significant differences at $p < 0.05$

Table 3 Main analytical parameters of the four vinegars

	BS	PS	PMS	
	H	*H*	*H*	*S*
pH	2.71b	2.65a	3.23c	3.24c
Total acidity (g/L of tartaric acid)	72.00a	72.75a	104.25c	93.38b
Acetic acid (g/L)	29.55a	84.79b	133.31c	132.09c
Ethanol (vol %)	nda	nda	nda	nda
Methanol (vol %)	0.11a	0.12a	0.11a	0.12a

Identical letters in row indicate no significant differences at $p < 0.05$

nd not detectable

Table 4 Analytical parameters related to the bioactive compounds and to the antioxidant activity of the eight wines

	BS		BMS		PS		PMS	
	H	*S*	*H*	*S*	*H*	*S*	*H*	*S*
Total phenolics (mg/L)	1889f	1899f	1417e	1379e	574b	954d	651c	114a
Gallic acid (mg/L)	5.00e	5.15e	3.79c	4.23d	3.84c	5.89f	2.40b	1.47a
Neochlorogenic acid (mg/L)	6.83e	6.09d	6.18d	6.69e	2.70a	2.66a	5.29c	4.22b
Chlorogenic acid (mg/L)	39.00d	52.74e	20.70b	31.59c	2.95a	8.16a	3.16a	3.13a
Vanillic acid (mg/L)	4.17d	5.59e	3.37c	3.53c	0.37a	0.62a	1.85b	0.77a
Caffeic acid (mg/L)	4.01e	3.45d	3.31d	2.65c	1.46a	3.00cd	2.01b	2.05b
Epicatechin (mg/L)	2.15e	2.44f	1.48c	1.56cd	1.72d	0.79b	0.73b	0.00a
Procyanidin A (mg/L)	0.00a	0.00a	92.18c	124.31d	0.00a	0.00a	12.73b	0.00a
Rutin (mg/L)	151.79g	147.94f	86.08e	71.30d	5.19b	6.18b	9.15c	0.73a
Quercetin (mg/L)	16.93e	20.21f	17.77e	6.53d	0.31a	1.24b	4.30c	0.40ab
Eriocitrin (mg/L)	10.49d	12.35e	5.88c	11.49de	0.85a	3.10b	0.00a	0.35a
Neoeriocitrin (mg/L)	538.36h	491.46g	390.95f	358.92e	156.08b	292.30d	167.04c	8.52a
Narirutin (mg/L)	20.73e	17.42d	11.31c	11.70c	3.16a	4.96b	3.97ab	5.21b
Naringin (mg/L)	642.91g	670.14h	502.53f	455.64e	270.51c	406.49d	253.02b	12.92a
Hesperidin (mg/L)	78.31g	107.37h	33.53d	65.87f	14.65a	30.45c	28.19b	36.22e
Neohesperidin (mg/L)	362.30g	350.93g	233.18f	220.12e	110.09b	184.73d	143.27c	32.89a
Didymin (mg/L)	5.78e	5.55de	4.25cde	2.84bc	0.29a	1.12ab	2.74bc	3.86cd
Hesperetin (mg/L)	0.28ab	0.27ab	0.22ab	0.48b	0.00a	1.92d	11.39e	0.96c
ABTS (% inhibition)	73.70f	67.73e	61.11d	55.01c	26.40a	33.52b	79.99g	73.54f
DPPH (% inhibition)	36.58h	35.84g	20.07c	24.27f	10.26a	12.09b	23.59e	22.25d
Brutieridin (mg/L)	695h	647g	365f	350e	97c	191d	68b	nda
Melitidin (mg/L)	386h	326g	200e	206f	73c	127d	38b	nda

Identical letters in row indicate no significant differences at $p < 0.05$

Table 5 Analytical parameters related to the bioactive compounds and to the antioxidant activity of the four vinegars

	BS	PS	PMS	
	H	*H*	*H*	*S*
Total phenolics (mg/L)	1953c	590b	615b	241a
Gallic acid (mg/L)	5.63c	4.08b	2.63a	2.62a
Neochlorogenic acid (mg/L)	5.83d	2.69a	4.96b	5.48c
Chlorogenic acid (mg/L)	58.51c	2.95a	7.53b	3.77a
Vanillic acid (mg/L)	6.03d	0.47a	1.31b	1.81c
Caffeic acid (mg/L)	3.64c	1.47a	1.39a	2.21b
Epicatechin (mg/L)	2.91d	1.97c	0.63b	0.00a
Procyanidin A (mg/L)	0.00a	0.00a	6.62b	9.43c
Rutin (mg/L)	146.31b	5.06a	1.76a	3.60a
Quercetin (mg/L)	8.62d	0.23a	0.95b	2.96c
Eriocitrin (mg/L)	13.20c	0.89b	0.31a	0.27a
Neoeriocitrin (mg/L)	513.30c	158.63b	157.42b	53.41a
Narirutin (mg/L)	18.24d	3.05a	6.54b	9.79c
naringin (mg/L)	700.56d	280.38c	245.56b	61.19a
Hesperidin (mg/L)	92.12d	12.15a	24.77c	19.55b
Neohesperidin (mg/L)	366.93d	113.88b	134.41c	63.51a
Didymin (mg/L)	9.82c	2.54b	2.36ab	1.73a
Hesperetin (mg/L)	1.12b	0.07a	15.54c	0.00a
ABTS (% inhibition)	55.39d	16.52a	31.40b	47.07c
DPPH (% inhibition)	21.80d	9.87a	18.88c	17.83b
Brutieridin (mg/L)	197d	46c	29b	nda
Melitidin (mg/L)	98d	31c	17b	nda

Identical letters in row indicate no significant differences at $p < 0.05$

Table 6 Volatile characterisation of the eight wines

	BS		BMS		PS		PMS	
	H	*S*	*H*	*S*	*H*	*S*	*H*	*S*
Ethyl acetate	2.10b	1.62a	2.40b	2.91c	6.18d	6.99e	20.83f	21.59g
1-Butanol-3-methyl	1.86b	1.38a	1.50a	4.44c	6.62e	5.41d	8.10g	7.86f
Bois de rose	1.04f	0.88e	0.54c	0.63d	0.15b	0.12b	0.00a	0.03a
Myrcene	0.73g	0.70g	0.46d	0.57f	0.27b	0.32c	0.15a	0.50e
Limonene	1.05f	0.68e	0.69e	0.38d	0.31c	0.26b	0.15a	0.25b
Eucalyptol	0.45e	0.46e	0.32d	0.47e	0.11bc	0.12c	0.09b	0.06a
Linalool oxide	1.07f	1.26g	0.65e	0.42c	0.41c	0.57d	0.31b	0.22a
Linalool	35.54b	33.58a	36.04b	42.91d	33.44a	45.13e	38.35c	43.61d
Fenchyl alcohol	3.88e	3.78e	3.54d	3.94e	2.32c	1.91b	1.80b	0.92a
Benzene 1 ethoxy 4 methyl	0.38f	0.43g	0.36e	0.24d	0.00a	0.03b	0.05c	0.04bc
Terpinen 4-ol	7.67e	8.34f	7.36e	5.64d	2.07a	3.63c	2.73b	2.06a
α-Terpineol	26.71de	29.82f	27.29e	25.87d	33.41g	14.52c	9.33b	6.67a
Citronellol	3.83c	5.42e	6.37f	3.84c	1.28ab	4.66d	1.52b	1.07a
Geraniol	4.11d	4.67e	5.64f	1.34b	1.41b	2.31c	0.21a	0.21a

The concentration of each compound is expressed as a percentage of the total volatiles (excluding ethanol)

Identical letters in row indicate no significant differences at $p < 0.05$

Table 7 Volatile characterisation of the four vinegars

	BS	PS	PMS	
	H	*H*	*H*	*S*
Isovaleric acid	2.08c	9.55d	0.50a	1.16b
Butyric acid	1.23a	2.75c	1.97b	6.98d
Benzaldehyde	0.27c	0.16b	0.47d	0.08a
Boise de rose oxide	3.06d	0.93c	0.72b	0.26a
cis-Dehydrolinalool oxide	0.91c	0.33b	0.30b	0.15a
Cineole	1.31c	0.23b	0.15a	0.09a
Limonene	1.26b	0.40a	2.04c	0.34a
Eucalyptol	1.40d	0.61c	0.26b	0.08a
Furan-2-carbaldehyde	3.34c	0.85b	0.79b	0.19a
cis-Linalool oxide	2.97c	2.81c	0.74b	0.50a
trans-Linalool oxide	2.24d	1.55b	2.01c	0.64a
Linalool	7.31b	2.96a	17.30c	28.96d
Fenchyl alcohol	4.64d	3.86c	1.95b	1.41a
Myrcenal	2.17d	1.02c	0.38b	0.20a
Endo-borneol	7.92c	0.35b	0.11a	0.48b
Terpinen-4-ol	9.93c	2.83a	3.23b	2.87ab
α-Terpineol	33.33c	11.97b	10.96a	11.36ab
Geranial ethyl-ether	0.64d	0.08a	0.34c	0.22b

The concentration of each compound is expressed as a percentage of the total volatiles (excluding acetic acid)

Identical letters in row indicate no significant differences at $p < 0.05$