

A study on relationships among chemical, physical, and qualitative assessment in traditional balsamic vinegar

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Abstract

Nineteen commercially available samples of aceto balsamico tradizionale (TBV, traditional balsamic vinegar) have been investigated, in order to study the relationships between their physical and chemical profiles and their sensory quality. Density, acidity, total phenols, furanic compounds, sugars, carboxylic acids and ABTS^{•+} radical scavenging assay were measured. Sugars, density and dry matter positively influence vinegar quality, while other parameters, such as acetic acid, have a negative influence. In addition, radical scavenging activity was not only correlated with phenolic content, as expected, but also with some quality parameters.

Also unexpected correlations between hydroxymethylfurfural and lactic acid and between vinegar quality, ash content and radical scavenging activity were found.

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1. Introduction

Aceto balsamico tradizionale (TBV, traditional balsamic vinegar) a typical product of Emilia-Romagna region (Italy), has become popular worldwide in recent years. In the past two decades, the physicochemical properties of TBV have been widely studied, although there has been little attempt to correlate composition with sensory quality (Chinnici, Masino, & Antonelli, 2003; Cocchi et al., 2006; Cocchi et al., 2004; Cocchi, Lambertini, Manzini, Marchetti, & Ulrici, 2002; Corradini et al., 1994; Giudici, Altieri, Masini, & Barbagallo, 1994; Plessi, Bertelli, & Miglietta, 2006; Plessi, Monzani, & Coppini, 1989; Sanarico, Motta, Bertolini, & Antonelli, 2003; Zeppa, Giordano, Gerbi, & Meglioli, 2002). In addition, TBV microbiology was also

accurately studied (De Vero et al., 2006; Gullo, Caggia, De Vero, & Giudici, 2006; Solieri, Landi, De Vero, & Giudici, 2006).

The quality of TBV is measured by a trained panel, using sensory evaluation. Qualified panellists participate in 15 panel tests per year, at least.

TBV samples are assessed for visual, olfactory and gustative qualities using a score card. Each mark has a multiplicative coefficient and the sum of scores gives a final value, which is used to assign TBV to its proper commercial class. Total acidity and density are also measured to complete the legal requirements (G.U., 2000). If a TBV does not reach the minimum score (240), it is excluded from labelling, while the three commercial classes are: from 241 to 269 (orange label), from 270 to 299 (silver label), and over 300 (gold label).

For all these reasons and for the considerable price of the product, TBV quality would deserve greater attention.

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Furthermore, many aspects of its composition and 41 nutritional characteristics (e.g. antioxidant power) have been neglected or occasionally studied. In particular, antioxidant power may be of some importance, because of the amount of phenolics and other antioxidant-related substances that have already been reported in vinegars (Plessi et al., 2006; Qingping, Wenyi, & Zonghua, 2006).

Nineteen samples of TBV from Reggio Emilia were evaluated for acid, sugar, and furanic compounds content, as well as other chemical and physical properties to give a large amount of information on TBV composition. In addition, radical scavenging activity (RSA) through the ABTS^{•+} test, was measured to evaluate the antioxidant properties of TBV, and for the first time was correlated with composition. The results obtained were evaluated with univariate and multivariate approaches, to explore the whole potential of the data set, showing the influence of some parameters on sensory quality.

2. Materials and methods

2.1. Chemicals

All reagents were of analytical grade. 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were obtained from Sigma–Aldrich (Milan, Italy).

2.2. Vinegar samples

Physicochemical properties of 19 TBVs, provided by the Consorzio tra i Produttori di Aceto Balsamico Tradizionale di Reggio Emilia (Reggio Emilia, Italy), previously evaluated by the official sensory panel as established by law, were analyzed (Table 1).

2.3. Radical scavenging activity

Radical scavenging activity (RSA) was assessed, using the ABTS free radical decolorisation assay, developed by Re et al. (1999) with some modification.

All the vinegar samples were diluted 200 times in water: ethanol (90:10). The ABTS^{•+} radical was generated by reacting ABTS aqueous solution (7 mM) with 2.45 mM potassium persulfate (K₂S₂O₈). The mixture was allowed to stand for 14–16 h in the dark at room temperature. This

stock solution was diluted with ethanol to obtain the ABTS working solution, with absorbance of 0.7 ± 0.02 AU at 734 nm.

An aliquot of 10 μ l of diluted vinegars was added to 1000 μ l of ABTS working solution.

The absorbance, monitored for 10 min, was measured at 734 nm and 37 °C with respect to a water:ethanol (90:10) blank. All measurements were performed in quadruplicate. The RSA of the samples were compared to that of Trolox by means of standard curves, obtained from known amounts of Trolox (from 0 to 0.25 mg/ml) and were expressed as Trolox equivalent antioxidant capacity (TEAC), defined as the millimolar concentration of a Trolox solution whose antioxidant capacity was equivalent to 1 kg of vinegar.

2.4. Total phenols

Total phenols (TP) were analysed according to the Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999) with some modifications. The TBV samples were diluted with distilled water to standardise the sugar content at 2 g/l (about 250 dilutions). In a 10 ml flask, 6 ml of distilled water, 0.3 ml of diluted vinegar and 0.5 ml of the Folin–Ciocalteu reagent were added and mixed. After 1 min, 2 ml of 15% aqueous sodium carbonate were added and the solution was made up to 10 ml with water. Finally, this solution was mixed and left to stand at room temperature for 120 min. Absorbance was read at 750 nm against a blank represented by a glucose solution (2 g/l in water) and compared with a standard gallic acid calibration curve. Results of triplicate analyses are given as mg of gallic acid equivalents (GAE) per kg of TBV.

3. Results and discussion

All data obtained, apart from the antioxidant and phenolic data, are reported in Table 2. Many considerations on these parameters were separately discussed in previous papers (Chinnici et al., 2003; Masino, Chinnici, Franchini, Ulrici, & Antonelli, 2005; Sanarico et al., 2003). There was a wide variability of values, indicating that large differences in TBV quality could be expected on commercial classification. Orange labelled vinegars are generally less dense, with a lower content in furanic compounds. Silver and gold labelled TBV were richer in sugars, dry residue, and contained less acetic acid.

For the same set of samples, the total phenolic content, RSA, and sensory score of each vinegar are given (Table 3). Values are broadly distributed and in some cases variability is large. For instance, the lowest TP content is 1460 mg/kg, while the highest is 5430 mg/kg.

Compared to musts or wines (Singleton, 1988), this latter value is very high. Initial must concentration and long ageing in wooden casks are the reasons for these figures.

RSA values were also very broad ranging from 14.5 mM to 58.2 mM Trolox equivalent antioxidant capacity

Table 1
Analytical methods applied to traditional balsamic vinegar samples

Analysis	Methodology	References
pH	Potentiometry	G.U. (1971)
Dry matter	Gravimetry	G.U. (1971)
Density	Densitometry	G.U. (1971)
Ashes	Gravimetric	G.U. (1971)
Acids and sugars	HPLC	Sanarico et al. (2003)
Furans	HPLC	Chinnici et al. (2003)

Table 2
Mean, standard deviation, minimum, and maximum values for physicochemical parameters of traditional balsamic vinegar

	Orange label (n = 8)			Silver label (n = 4)			Gold label (n = 7)		
	Mean ± STD	Min	Max	Mean ± STD	Min	Max	Mean ± STD	Min	Max
Citric acid (g/100 g)	0.083 ± 0.027	0.050	0.141	0.023 ± 0.026	0.000	0.047	0.075 ± 0.071	0.000	0.208
Tartaric acid (g/100 g)	0.506 ± 0.092	0.394	0.661	0.608 ± 0.140	0.487	0.769	0.575 ± 0.151	0.379	0.753
Gluconic acid (g/100 g)	0.475 ± 0.295	0.085	0.922	0.617 ± 0.268	0.268	0.895	1.518 ± 2.311	0.111	6.681
Malic acid (g/100 g)	0.836 ± 0.372	0.436	1.47	0.768 ± 0.193	0.590	0.976	0.703 ± 0.142	0.545	0.968
Succinic acid (g/100 g)	0.993 ± 0.339	0.679	1.77	0.970 ± 0.054	0.918	1.02	1.166 ± 0.230	0.894	1.48
Lactic acid (g/100 g)	0.055 ± 0.012	0.039	0.071	0.338 ± 0.553	0.059	1.167	0.065 ± 0.019	0.045	0.099
Acetic acid (g/100 g)	2.65 ± 0.353 ^b	2.13	3.08	1.95 ± 0.129 ^a	1.85	2.13	1.99 ± 0.450 ^a	1.21	2.51
Glucose (g/100 g)	23.8 ± 1.01 ^a	22.7	25.6	26.5 ± 0.97 ^b	25.5	27.8	27.6 ± 2.40 ^b	24.1	30.9
Fructose (g/100 g)	21.4 ± 1.23 ^a	19.2	22.8	23.4 ± 1.20 ^a	22.2	25.0	26.5 ± 2.72 ^b	24.0	31.6
Dry residue (%)	55.5 ± 1.6 ^a	53.0	58.0	66.5 ± 1.7 ^b	65.0	69.0	66.7 ± 4.5 ^b	58.0	71.0
Ashes (%)	0.675 ^{ab} ± 0.125	0.470	0.920	0.618 ^a ± 0.127	0.440	0.740	0.859 ^b ± 0.191	0.640	1.180
pH	2.49 ± 0.12	2.36	2.65	2.48 ± 0.05	2.43	2.53	2.40 ± 0.13	2.22	2.60
°Brix	57.1 ^a ± 1.6	55.0	60.0	67.9 ^b ± 2.4	66.5	71.5	70.0 ^b ± 2.7	66.0	73.0
Density (g/l)	1.27 ± 0.009 ^a	1.26	1.29	1.33 ± 0.023 ^b	1.32	1.37	1.35 ± 0.015 ^b	1.33	1.37
Total organic acids (eq/kg)	0.843 ± 0.122	0.665	1.058	0.757 ± 0.083	0.688	0.861	0.804 ± 0.100	0.695	0.954
Total acidity (eq/kg)	0.855 ± 0.104	0.700	1.010	0.783 ± 0.071	0.720	0.870	0.790 ± 0.101	0.600	0.880
Furoic acid (mg/kg)	38.0 ± 7.0	25.4	46.9	46.0 ± 5.4	39.2	50.7	44.1 ± 26.4	13.3	94.6
HMF (mg/kg)	2906 ± 497 ^a	2387	3809	3881 ± 1031 ^b	3107	5356	3444 ± 382 ^{ab}	3056	4039
Furfural (mg/kg)	28.9 ± 17.8	4.9	65.7	40.2 ± 19.1	18.0	64.5	40.5 ± 20.5	11.8	65.0
AMFA (mg/kg)	9.9 ± 18.8	0.0	47.7	70.5 ± 53.9	0.0	130.0	51.7 ± 72.8	0.0	188.1

In the same row, means with different superscripts are significantly different values ($p < 0.05$); STD: standard deviation; n: number of samples; min: minimum value; max: maximum values; HMF: 5-hydroxymethylfurfural; AMFA: 5-acetoxymethyl-2-furaldehyde.

Table 3
Radical scavenging activity (RSA), total phenolics (TP) and sensory scores of traditional balsamic vinegar samples

Sample	RSA ^a (n = 4) Mean ± STD	TP ^b (n = 3) Mean ± STD	Scores
1	22.9 ± 2.49	3327 ± 213	292
2	23.1 ± 0.89	2938 ± 222	315
3	43.1 ± 2.68	3253 ± 313	294
4	38.9 ± 1.62	2707 ± 223	293
5	50.4 ± 2.89	3712 ± 634	317
6	37.3 ± 3.01	2905 ± 452	297
7	19.3 ± 0.41	1876 ± 228	248
8	53.6 ± 3.73	4285 ± 306	315
9	49.6 ± 3.57	3645 ± 345	301
10	21.0 ± 1.50	2421 ± 285	240
11	18.6 ± 1.26	2053 ± 216	253
12	20.9 ± 1.68	1613 ± 236	256
13	17.6 ± 1.16	1879 ± 236	256
14	14.5 ± 1.44	1455 ± 177	258
15	32.4 ± 1.05	3272 ± 536	265
16	19.8 ± 2.06	2603 ± 239	265
17	18.1 ± 0.48	1976 ± 140	311
18	51.9 ± 2.55	3792 ± 272	314
19	58.2 ± 4.88	5433 ± 472	332

^a Expressed as mM/Trolox equivalent antioxidant capacity.

^b Expressed as mg/Kg Gallic acid equivalents.

(TEAC). Due to the lack of published data on TBV antioxidant activity, it is not possible to compare our results with findings from other authors. However, TBV has a very high RSA, if compared with data published by Pellegrini et al. (2003). These authors reported values of 3.15 mM TEAC in red wine vinegar, and 1.52 and 12.1 in white and red wines, respectively. In addition, Alonso, Remedios,

Rodriguez, Guillen, and Barroso (2004) found up to 6 mM TEAC for aged or non-aged sherry vinegars. TBV antioxidant activity is also higher than blackberry (24.2 mM TEAC) or espresso coffee (36.5 mM TEAC) (Pellegrini et al., 2003). Apart from phenolics, substances that could be responsible for such a high radical scavenging activity could be melanoidins from the Maillard reaction (Quingping et al., 2003), and/or wood phenolics.

Correlation analysis carried out on the whole data set shows many correlations (Table 4). Acetic acid was negatively correlated with °Brix, density, sensory score, gluconic acid, dry residue, RSA, and TP. Particularly interesting is the good correlation of acetic acid vs. score, as a consequence of its high impact on flavour. High acetic acid content is typical of ordinary wine vinegar, and tends to hide many of the subtle notes of TBV.

3.1. Furanic compounds were not widely correlated with other parameters

Hydroxymethylfurfural (HMF) showed good positive correlations with dry matter, °Brix, and density. Moreover HMF showed a positive correlation with lactic acid, which has no other significant correlations. In wine, lactic acid can be produced by yeasts (by far mainly D-enantiomer), as a side-product of alcoholic fermentation from pyruvic acid, or by lactic bacteria from malic acid (malolactic fermentation that yields only L-enantiomer). The high amounts of malic acid in all TBV samples excludes any malolactic bacteria activity. Moreover, previous studies on the enantiomeric abundance of this acid in TBV and other vinegars (Plessi et al., 1989) revealed a sharp predominance

Table 4
Correlation matrix of the data set

	Citric acid	Tartaric acid	Gluconic acid	Malic acid	Succinic acid	Lactic acid	Acetic acid	Glucose	Fructose	Dry residue	Ash	pH	°Brix	Density	Total organic acids	Total acidity	Quality Score	Furoic acid	HMF	Furfural	AMFA	RSA	Total polyphenols	
Citric acid	1																							
Tartaric acid		1																						
Gluconic acid			1																					
Malic acid				1																				
Succinic acid					1																			
Lactic acid						1																		
Acetic acid							1																	
Glucose								1																
Fructose								0.845	1															
Dry residue										1														
Ash											1													
pH												1												
°Brix													1											
Density														1										
Total organic acids															1									
Total acidity																1								
Quality Score																	1							
Furoic acid																		1						
HMF																			1					
Furfural																				1				
AMFA																					1			
RSA																						1		
Total polyphenols																							1	

For sake of clarity, correlation coefficients (R) with $p < 0.05$ are reported.

HMF, hydroxymethylfurfural; AMFA, 5-acetoxymethyl-2-furaldehyde; RSA, radical scavenging activity.

of the L form. In the literature, lactic acid has already been reported as a side-product of Maillard reaction (Davidek, Robert, Devaud, Vera, & Blank, 2006). These authors suggested a α -dicarbonyl cleavage of glucose on heating, as possible pathway for lactic acid formation. This mechanism explains lactic acid correlation with HMF, and suggests that its occurrence in our samples is partially independent from biological origin. In addition, the absence of significant correlations of lactic acid vs. malic acid further supports its chemical origin. Thus, the D isomer could be produced by yeasts at the beginning of the ageing process, while the L-enantiomer may come from the cleavage of the 1-deoxy-2,4-hexulose as demonstrated by Davidek et al. (2006), justifying the prevailing sugar cleavage way for the formation of this acid. At the moment, the actual origin of this acid is still under investigation.

Sugars have good positive correlations with concentration parameters, such as density, and hence °Brix and dry matter. Moreover, a negative correlation with acids was found. The susceptibility to degradation of sugars in acidic media is the probable reason, and the high negative *R*-value for fructose seems to confirm this behaviour. This sugar is less stable than glucose under acid conditions as it can undergo to a double enolisation in the first step of degradation (Belitz & Grosch, 1999).

RSA shows many correlations with other parameters. Its correlation with TP is very high, a consequence of the importance of polyphenols on the antioxidant properties of food (Rice-Evans, Miller, & Paganga, 1997) (Fig. 1). The positive correlation of TP with concentration parameters is the obvious response to their increase occurring during the process, and high correlation with quality score is an indirect consequence of these considerations.

Ash vs. RSA is one of the most interesting correlations for many reasons. Firstly, ash is not correlated with any concentration parameters, but only with score and with TP. It is very likely that some metals, especially transition metals such as Cu and Fe, play a fundamental role as redox catalysts. Literature reports only few data on metal content in TBV (Cocchi et al., 2004; Corradini et al., 1994). How-

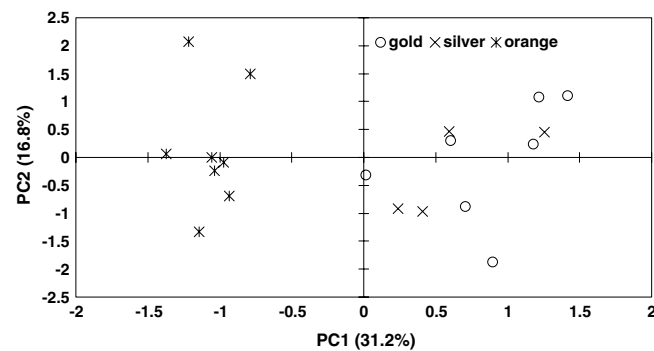


Fig. 2. Principal component analysis of traditional balsamic vinegar samples: plot of the first two principal components (PC1 and PC2) with the explained variance in parentheses.

ever, it is reasonable to assume that Cu is not very important because of its low amount and because its concentration does not vary during aging (Cocchi et al., 2004), probably as a consequence of its precipitation. On the contrary, Fe undergoes a considerable increase during ageing, which could reach up to 10 times the original content. Cocchi et al. (2004) found up to 156 mg/kg of Fe in TBV, a very high concentration, compared to must and wine. In wine, Fe concentration is usually lower than 1 mg/l (Buldini, Cavalli, & Sharma, 1999). The role of Fe concentration on the antioxidant properties of TBV may require greater attention in the future.

PCA results are presented in Fig. 2. The total variability of the sample set (62.4%) is explained by the first 3 PCs. Dry matter, °Brix, and density, as already seen with ANOVA, are positively correlated with the first component (PC1, concentration), and sugars (glucose and fructose) are most important substances able to influence on these parameters. However, their relative low loading value (0.6) suggests that some other components, which were not measured, contribute to dry matter composition. Acetic acid has a negative weight on this component, and explains 31.2% of sample set variability. Along this component, samples are grouped in 2 clusters, with low score TBVs (orange label) in the negative part and with high score TBVs (silver and gold labels) in the positive part.

PC2 (acidity) contributes to a further 16.8% of variance, but samples are scattered along this component with no evident pattern. In this case the role of each acid seems negligible, but total acidity, acid sum, and pH contribute to the variability expressed by PC2.

Finally, ash explains a further 14.4% on the PC3.

4. Conclusions

The quality of TBV relies mainly on sugar content, density, °Brix, and dry residue and secondly on acidity. However, as some parameters have no effect on quality, or are highly correlated with other parameters, there is no reason to perform all the analyses reported in this paper. For instance, simple determinations as dry residue or density do not require other analyses, such as sugar content or

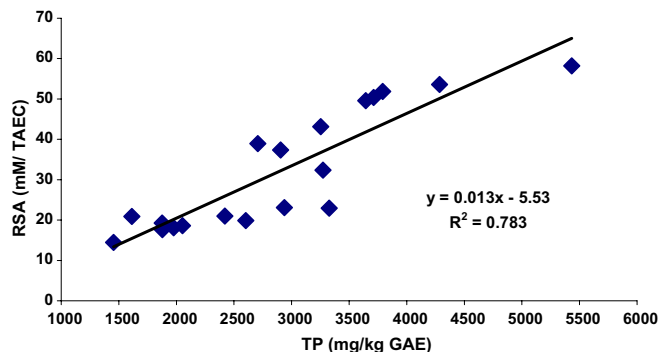


Fig. 1. Correlation between total phenols (TP), expressed as Gallic acid equivalents (GAE), and radical 254 scavenging activity (RSA) expressed as Trolox equivalent antioxidant capacity (TEAC) in traditional balsamic vinegar.

°Brix. Similar considerations can be applied to acidity and single acid quantifications, with the exception of acetic acid for its negative correlation with main TBV quality parameters. The quantification of this acid, along with pH measurement, appears to be sufficient to support the information that derives from acidity parameters.

On the other hand, ash determination seems to be more important than is usually considered. A deeper knowledge of single metal content could be of some support to TBV characterisation and quality evaluation.

Also, RSA seems to be a promising field of investigation for the very high values exhibited by the analysed samples. This parameter is important not only for quality investigation, but also for its nutritional implication. HMF and TP can complete the set of analyses to characterise and discriminate TBVs. All other parameters seem to be redundant, time and money consuming, with no real contribution to TBV quality determination.

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