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A study of the relationships among acidity, sugar and furanic compound concentrations in set of casks for Aceto Balsamico Tradizionale of Reggio Emilia by multivariate techniques

Francesca Masino [a], Fabio Chinnici [b], Gian Carlo Franchini [a], Alessandro Ulrici [a], Andrea Antonelli [a,*]

[a] *Dip. Interdisc. Sc. Agrarie, Via J.F. Kennedy 17, 42100 Reggio Emilia, Italy*
[b] *Dip. Sc. Alim., Via Fanin 40, 40127 Bologna, Italy*

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Abstract

"Aceto Balsamico Tradizionale" (ABT) is gaining increasing attention due to its peculiar characteristics, not only within the production area. A first approach to understanding the complex ageing process was performed by studying ABT furanic compounds, quantified by HPLC: hydroxymethylfurfural, furoic acid, furfural, and 5-acetoxymethylfurfural (HMF, FA, Fal, AMFA). Also, other parameters, expressing the acidic and sugar contents (pH, total acidity and °Brix), were quantified.

Furanic compounds arise during the must concentration process. Moreover, they tend to rise on ageing, as a consequence of the water loss during the process. However, due to the great reactivity of these substances, which rapidly evolve into other compounds, differences in the accumulation kinetics are likely.

While the separate analyses of the single variables did not lead to any significant contribution in the understanding of the phenomena involved in the product transformation, principal component analysis showed a common trend on ageing for all the studied sets.

Keywords: Aceto balsamico tradizionale; Traditional balsamic vinegar; ABT; Chemometrics; Principal component analysis; Vinegar; 5- Hydroxymethylfurfural

1. Introduction

The "Aceto Balsamico Tradizionale" (traditional balsamic vinegar) of Reggio Emilia (ABT) is a typical product of the Emilia Romagna region in the north of Italy. It is produced by slow ageing of cooked must from local grapes, for at least 12 years (G.U., 1986) in sets of casks of decreasing volume and made of different woods. Once a year, a portion of the final product is drawn from the smallest barrel (cask No. 1 in Fig. 1), which is refilled with the content of the previous one (cask No. 2). The practice is carried out up to the largest barrel (cask No. 5), which is eventually filled with fresh cooked must. On ageing, the product undergoes a further slow concentration process by water evaporation through the staves. Moreover, in the first barrels, an alcoholic oxidation and a subsequent acetic oxidation occur. In the following barrels, contents (a_w, acetic acid concentration, acidity) prevent any further biological activity. The final result is a thick dark syrup, which is very appreciated as a sauce for adding to many different gastronomic products (e.g., parmesan, meat, salad, strawberries.) with a characteristic and pleasant aroma. Further details about the ABT production technique can be found in previous literature

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[*] Corresponding author. Tel.: +39 522 300531; fax: +39 522 301482.
E-mail address: andrea.antonelli@unimo.it (A. Antonelli).

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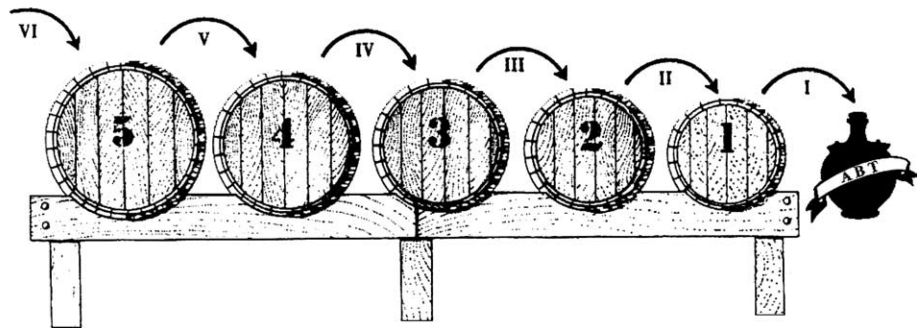

Fig. 1. A typical set of barrels for "Aceto Balsamico tradizionale" (ABT) production. Arabic numerals identify each cask, while Roman numerals indicate the sequence of the drawing procedure.

(Cocchi, Lambertini, Manzini, Marchetti, & Ulrici, 2002). In a previous paper (Antonelli, Chinnici, & Masino, 2004), some of us demonstrated that must concentration was responsible for a variety of sugar degradation products, the most important being hydroxymethylfurfural (HMF). This substance is formed in many heat-processed products (Lee & Nagy, 1990; Morales, Romero, & Jimenez-Pérez, 1992). The heat concentration process of grape must is the main HMF source, but there is evidence that this compound, and its congeners, slowly increases at room temperature as also in minimally heat-processed foods. Honey (Salinas, Mansilla, & Nevado, 1991), fruit juices (Maijares, Park, Nelson, & McIver, 1986) and wine (Williams, Humphreys, & Reader, 1983) are a few examples where this formation occurs. Besides HMF, must cooking yields some other furanic congeners: furoic acid (FA), furaldehyde (Fal) and acetoxymethylfurfural (AMFA) (Antonelli et al., 2004). Until now, the variations in concentration of these compounds during ageing and along the cask sets have not been described in the scientific literature.

In order to interpret such a complex process, we used a multivariate approach, i.e. principal component analysis (PCA), which allows concentration of the information of the data set into a reduced number of variables (Massart et al., 1997).

In particular, the evolution of some main composition parameters (pH, total acidity, °Brix, along with furanic compounds) was studied in sets of barrels used for ABT production. PCA showed an ageing pattern common to almost all the analysed sets, but it also emphasised the peculiarity of some of them.

2. Materials and methods

2.1. Samples

Seven sets (composed of five casks each), representative of the ABT production area, were chosen. The sets were managed strictly, following the classical method described in Section 1. A 100 ml sample aliquot was taken from each barrel at the moment of ABT drawing, as described previously.

According to tradition, barrels are numbered as shown in Fig. 1, i.e. from the smallest one to the largest one: cask No. 1 contains the oldest product (ABT), while in cask No. 5 the cooked must starts its ageing.

2.2. Physical and chemical determinations

Undiluted samples were used for pH and °Brix measures, which were carried out with a pH meter and a refractometer, respectively. Total acidity was measured by the titrimetric method for wine and must analyses.

FA, HMF, Fal and AMFA were determined by an HPLC method previously published (Chinnici, Masino, & Antonelli, 2003), using a cation exchange column isocratically operating and diode array detection.

Multivariate analysis of data was carried out in the MATLAB ver. 6.5 environment (MathWorks Inc., Natick, MA, USA), using the routines of the PLS Toolbox 2.1 (Eigenvector Inc., Natick, MA, USA).

3. Results and discussion

Table 1 summarises the analytical results of the considered samples. Sugar concentration (°Brix) ranged from 21.3 (cask 5, set A) to 72.5 (cask 1, set F). This parameter showed an increase, on ageing, for sets A, D, E, F, and G, but the same trend was not observed for sets B and C. When considered separately, the values of pH, which varied from 2.02 (cask 1, set F) to 3.50 (cask 3, set F), and total acidity, that ranged from 1.76 g/100 g (cask 5, set A) to 6.72 g/100 g (cask 1, set A), did not yield useful information.

As a consequence of sugar degradation, must cooking generates a great amount of furanic compounds (Antonelli et al., 2004). In many cases, HMF was above 3 g/kg. In five sets (A, D, E, F, and G), HMF increased regularly along the cask series, reaching final concentrations up to six times those of the barrel containing the youngest product (from 246 to 1441 mg/kg for set A).

Table 1
Results of the determinations performed on the samples

Set	Cask	pH	Total acidity	°Brix	FA	HMF	AMFA	Fal
A	5	2.58	1.76	21.3	6	246	n.d.[a]	n.d.
A	4	2.55	2.68	36.3	7	471	n.d.	n.d.
A	3	2.48	4.24	45.3	18	933	5	7
A	2	2.48	4.95	53.5	30	1091	24	10
A	1	2.36	6.72	60.0	49	1441	55	16
B	5	2.64	2.65	58.2	10	3420	41	7
B	4	2.84	2.84	65.5	13	2840	49	8
B	3	2.52	4.23	64.0	27	3221	65	12
B	2	2.45	3.81	64.4	30	3173	70	12
B	1	2.68	3.92	67.4	27	3181	85	9
C	5	2.77	4.11	40.0	12	2684	31	5
C	4	2.46	4.94	57.0	31	2485	56	12
C	3	2.78	4.26	57.0	25	3083	46	15
C	2	2.46	4.35	56.3	25	3000	70	13
C	1	2.43	4.71	69.5	34	3213	104	15
D	5	2.94	1.81	49.2	8	1189	10	5
D	4	2.71	3.38	50.7	18	1382	10	11
D	3	2.82	4.76	52.0	21	1814	14	16
D	2	2.61	5.62	55.0	25	2160	36	17
D	1	2.67	5.50	58.0	27	2398	60	20
E	5	2.88	2.11	46.5	16	1081	5	8
E	4	2.84	5.23	53.5	21	1688	14	13
E	3	2.56	4.03	58.7	21	2186	27	13
E	2	2.95	4.08	63.0	21	2517	29	14
E	1	2.40	3.78	65.0	23	2644	50	14
F	5	3.11	3.41	47.0	14	1140	8	2
F	4	2.93	3.42	54.7	22	1737	13	17
F	3	3.50	3.11	62.4	14	2182	23	20
F	2	2.84	3.07	67.5	19	2747	15	22
F	1	2.02	3.92	72.5	32	3249	69	28
G	5	2.66	2.42	33.3	9	645	n.d.	5
G	4	2.79	5.32	47.0	32	1265	17	7
G	3	2.58	5.32	53.3	28	1559	33	11
G	2	2.60	4.97	61.0	31	1936	26	9
G	1	2.67	4.10	67.5	37	2563	56	10

Values are expressed as mg/kg ABT, except for total acidity (g/100 g product), pH and °Brix. Within each set, the casks are numbered from no. 5, which identifies the largest and youngest one, to no. 1, for the smallest and oldest one. FA, furoic acid; HMF, 5-hydroxymethylfurfural; AMFA, acetoxymethylfurfural; Fal, furfural.

[a] n.d.: not detected.

By contrast, in sets B and C, the HMF content was almost constant.

In order to estimate whether the observed variations were due only to product concentration during ageing or also to sugar degradation, the ratios between the initial and the final °Brix and HMF concentration values were considered (Fig. 2). Except for sets B and C, the solute concentration ratios (which correspond to the °Brix ratios) showed a narrower variation than the HMF ratios. These data suggest that sugar degradation may also occur during ageing and that HMF formation is not limited to the step of must cooking. In addition, it should be noted that the ABT production procedure requires that the sets must be placed at the last floor, just under the roofs, to be exposed to the climate variations. Thus, during summer, the temperature can easily reach 40 °C on even more, which is sufficient to promote furan formation. The other furanic congeners did not follow this pattern (Table 1), with FA showing the most irregular behaviour.

As a first approach, in order to evaluate the relationships among the discussed variables, the correlation matrix was calculated (Table 2). Only correlations with a squared correlation coefficient $R^2 > 0.500$ (corresponding to $|r| > 0.708$) were considered, restricting the number of significant correlations to six. A significant correlation was found between total acidity and FA. This latter variable was also found to be correlated with the cask number, which, in turn, is correlated with the concentration of the product (°Brix). However, neither of these two correlations, even if statistically significant, can be judged satisfactory for predicting the cask number (i.e. the ageing of the product) with efficient accuracy. The concentration of the product was found to be correlated also with HMF and AMFA. This fact is very likely due to the concentration of the solutes in

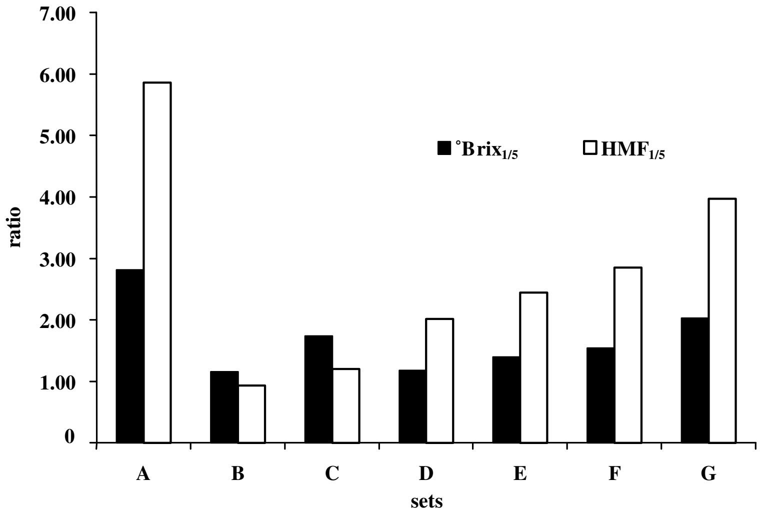

Fig. 2. Ratios between the values of cask 1 (ABT) and cask 5 (product at the beginning of the process) for total soluble solids (°Brix) and hydroxymethylfurfural (HMF) in all sets.

Table 2
Correlation matrix

	Cask no.	pH	Total acidity	°Brix	FA	HMF	AMFA	Fal
Cask no.	1.0000							
pH	0.4351	1.0000						
Total acidity	−0.5665	−0.3233	1.0000					
°Brix	***−0.7159***	−0.1465	0.3448	1.0000				
FA	***−0.7404***	−0.4668	***0.8020***	0.5932	1.0000			
HMF	−0.4851	−0.1851	0.1945	***0.7959***	0.3434	1.0000		
AMFA	−0.6745	−0.4639	0.3964	***0.7138***	0.6196	***0.8049***	1.0000	
Fal	−0.6372	−0.1319	0.4093	0.6970	0.5005	0.5311	0.4192	1.0000

Significant correlations are marked with bold and italic characters. FA, furoic acid; HMF, 5-hydroxymethylfurfural; AMFA, acetoxymethylfurfural; Fal, furfural.

general, which occurs during ageing, but it can also be connected with slow acid degradation of the reducing sugars. A significant correlation has also been found between HMF and AMFA, as an obvious consequence of their biochemical relation. On the other hand, AMFA concentration relies, not only on HMF concentration, its parent compound, but also on bacterial activity, the source of acetic acid.

Such a univariate approach, based on the analysis of the correlation matrix, can be a useful tool for a first screening of the data set, able to support the more obvious correlations between couples of variables. However, for a more complete analysis of the data matrix, and in order to gain a deeper understanding of the complex phenomena involved in ABT production, more powerful multivariate analysis methods are needed. In fact, PCA enables the systematic variation in the data set to be represented by a reduced number of factors, called principal components (PCs), whose number depends on the real dimensionality of the data set itself. From the PCA analysis of the autoscaled data matrix, composed of the values of the seven chemical parameters (variables) measured in each of the 35 casks (objects), only the first two PCs were significant, explaining 74.4% of the total variance.

All the studied parameters except pH contributed to PC1 (56.0% explained variance) with almost the same positive weight, as shown in the biplot of PC1 vs. PC2 (Fig. 3), where they lie on the right hand of the graph. The negative influence of pH (left side of Fig. 3) was somewhat expected, since furanic compound synthesis is enhanced by low values of this parameter, and the link among pH, total acidity and °Brix was an obvious effect of the concentration process.

In order to give a clearer interpretation of PCA results, only the PC1 scores vs. the sample numbers are reported (Fig. 4). In this figure, casks of the same set are joined by a solid line and identified by the same symbol. The observed trends increase almost monotonically, with the unique exceptions within sets B and C, characterised by very limited decreases of the score values. These trends are consistent with the ageing process of ABT production, which is characterised by a gradual increase of the chemical species concentrations and by the lowering of pH.

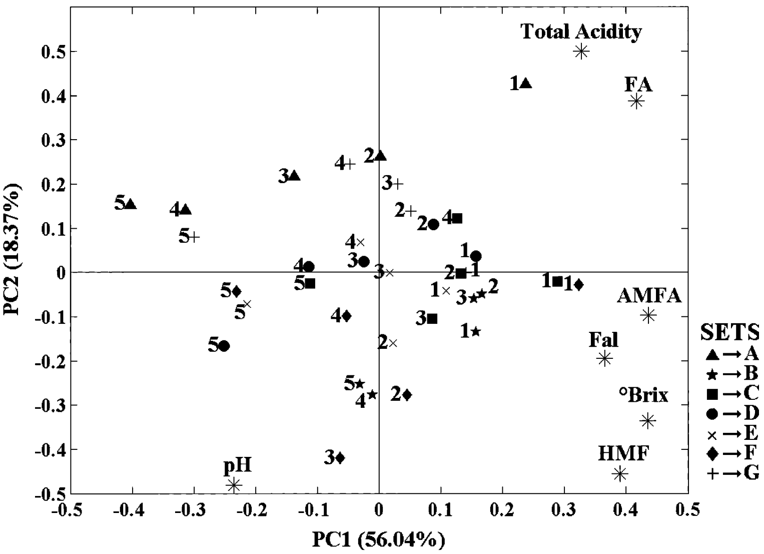

Fig. 3. Biplot of the first two principal components. The different symbols (triangle, star, square, circle, x-mark, rhombus, plus sign) are referred to the different sets, as reported in the legend, with the exception of the asterisks, that are referred to the original variables (FA, furoic acid; HMF, 5-hydroxymethylfurfural; AMFA, acetoxymethylfurfural; Fal, furfural). The numbers identify the cask order (from 1 for the final product, up to 5 for the initial product – see Fig. 1).

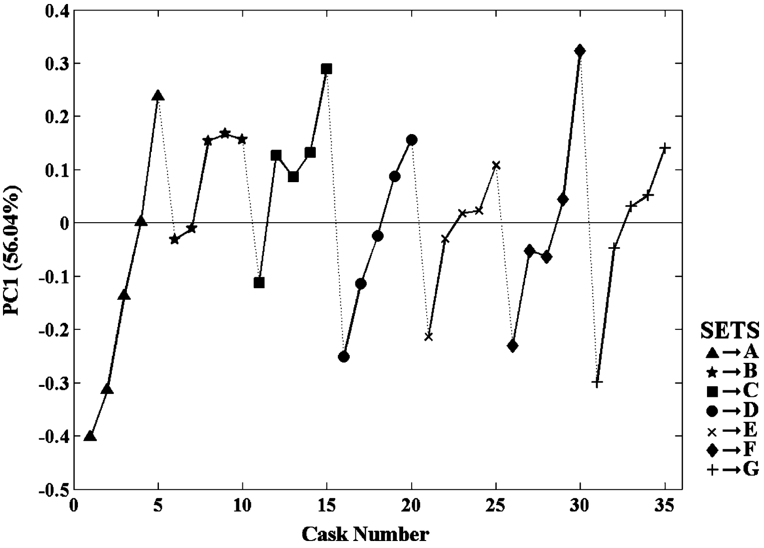

Fig. 4. Scores plot of the first principal component (PC1) versus the sample number. The same symbol and a continuous line identify casks of the same set.

While, in some cases (in particular for sets A and D), there is a linear increase with ageing of the PC1 scores within the sets, for other sets the scores values behave more as *n*-level variables (e.g. two levels for set B). This departure from linearity for some sets suggests that a higher number of descriptors should be used to properly model the ageing of the product by means of linear regression techniques, such as PLS. To confirm this hypothesis, a PLS model of the cask number as a function of the (autoscaled) measured variables (pH, total acidity, °Brix, HMF, FA, AMFA, Fal) was calculated and, as expected, it did not give satisfactory results. The values of the squared correlation coefficient and of the squared correlation coefficient in cross-validation (calculated by the leave one out technique) for the best calibration model (one latent variable, according to the minimum value of the root mean square error in cross-validation, RMSECV) were found to be quite low: $R^2 = 0.6867$ and $R^2_{\mathrm{CV,LOO}} = 0.6374$. It is interesting to note that the loadings vector of the unique selected latent variable of this PLS model is very similar to the loadings vector of PC1 of the PCA model. This fact confirms that the greatest contribution to the variance of the analysed data set is ascribable to the inter-cask within-sets variability.

As for as PC2 is concerned (18.7% explained variance), from Fig. 3 it can be seen that its variation is essentially due to pH, °Brix and HMF (with positive sign), and to total acidity and FA (with negative sign). Looking at the distribution of score values along PC2, it can be seen that they do not show a common trend with the cask number for all the sets, as was the case for PC1, but rather some distinctions among the different cask sets can be observed. In fact, the analysed sets tend to split off along PC2, independently of the cask number. Sets A and G always have positive PC2 scores, sets B and F always have negative values, while the remaining sets (C, D, E) have both positive and negative values and, on average, are closer to zero. This distribution can be partly ascribed to the different ages of the cask sets. In fact, the oldest sets B and F (28 and 70 years old, respectively), lie in an opposite position with respect to set A, which is the youngest one (12 years old), while the 20-year-old set D and E, are in an intermediate position. Unfortunately, the ages of sets C and G are not available, this fact limiting the possibility of further investigation.

Furanic compounds and °Brix lie in the same quadrant of Fig. 3, with the exception of FA, which is close to total acidity in the upper right quadrant. The common origin of furanic compounds is responsible for their homogeneous distribution that is strictly related to sugars and hence to °Brix. FA is generally assumed to come from ascorbic acid (Sawamura, Takemoto, Uked, & Kusunose, 1994), which is rather scarce in grape must. However, the proposed pathway suggested the presence of a L-xylosone intermediate, which could also originate though a more likely sequence involving hexose or pentose sugars, more abundant than ascorbic acid in the analysed food matrix. Anyway, the different origin of FA justifies its high correlation with total acidity in opposition to HMF, the main product of sugar degradation. The opposite position of pH and total acidity, with respect to the axes of origin is the natural consequence of the fact that these two variables are negatively correlated. This was not evident from the correlation matrix ($R_{\text{pH/total acidity}}$; = −0.3233, see Table 2) due to the high amount of noise in the data, but it emerges when considering the restricted space of the first two PCs, where only systematic variation is maintained.

The interpretation of the spatial distribution of the samples is not straightforward. However, a careful inspection shows that, for five of the seven sets, the youngest casks, identified by number 5, lie quite separately on the left side of the biplot, together with cask number 4 of the youngest set (set A). On the basis of these few analytical parameters, this observation suggests that it is possible to discriminate products at the beginning of ageing. By contrast, when the oldest product are considered, i.e. ABT samples (labelled with number 1), they lie on the opposite side of the plot, and they can be roughly grouped into three "subclasses". The first one is composed of ABT of sets C and F, which are very similar and are separated from the products of intermediate age. The second "subclass" is that of the four ABTs from sets B, D, E, and G, which lie in the same region as occupied by other unripe samples of various sets. Finally, ABT of set A is completely apart from all the other samples, including those of its set, mainly because of its high values of total acidity and FA concentration.

4. Conclusions

The great amount of random variation in the measured data made the use of simple univariate analysis techniques quite meaningless. In fact, only few correlations were found to be statistically significant, whose rationalisation in terms of the underlying chemical phenomena did not help very much in improving the knowledge of the investigated product.

Conversely, the use of PCA allowed ageing of the product to be identified within each production set as the first source of systematic variation of the analysed parameters. However, the determination of only seven parameters did not allow prediction of ageing of the product (i.e. its cask number) with sufficient accuracy. Further investigation would be helpful for achieving this aim. Finally, PCA on the data considered for this preliminary study suggests that these investigated variables would also contain information related to the age of the entire set of barrels.

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