JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Chemical and Biochemical Transformations during the Industrial Process of Sherry Vinegar Aging

VICTOR PALACIOS,*,† MANUEL VALCÁRCEL,‡ ILDEFONSO CARO,† AND LUIS PÉREZ†

Department of Chemical Engineering, Food Technology and Environmental Technology, Faculty of Sciences, University of Cadiz, Campus Río San Pedro, Apdo. 40, Puerto Real, 11510 Cádiz, Spain, and Department of Research and Development, Pedro Domecq, Jerez de la Frontera, Spain

The work described here concerns a study of the chemical and biochemical transformations in sherry vinegar during the different aging stages. The main factors that contribute to the nature and special characteristics of sherry vinegar are the raw sherry wine, the traditional process of acetic acid fermentation in butts (the solera system), and the physicochemical activity during the aging process in the solera system. A number of chemical and biochemical changes that occur during sherry vinegar aging are similar to those that take place in sherry wine during its biological activity process (where the wine types obtained are fino and manzanilla) or physicochemical activity process (to give oloroso wines). Significant increase in acetic acid levels was observed during the biological activity phase. In addition, the concentrations of tartaric, gluconic, succinic, and citric acids increased during the aging, as did levels of amino acids and acetoin. A color change was also produced during this stage. Glycerol was not consumed by acetic acid bacteria, and levels of higher alcohols decreased because of the synthesis of acetates. On the other hand, in the physicochemical phase the microbiological activity was lower. Concentrations of some cations increased because of evaporation of water through the wood. A color change was also produced in this stage. Concentrations of different amino acids decreased because of reaction with carbonyl compounds. A precipitation of potassium with tartaric acid was also observed.

KEYWORDS: Sherry vinegar; aging stages; chemical changes; biochemical changes

INTRODUCTION

Wine vinegar is an enological product that achieves, depending on the type produced, a distinctive personality that engenders a high degree of appreciation among consumers. Sherry vinegar is one of the most renowned products of this type in the world. The long enological tradition associated with the regions around Jerez de la Frontera (Spain) has led to the development of a very special vinegar of premium quality, which has subsequently become widely appreciated in overseas markets. Production of such vinegar has reached levels of nearly four million liters per year. The special characteristics of this vinegar, which have been described in many studies (*1*−*3*), along with its positive image, has led to the creation of denomination of origin "sherry vinegar". However, despite the long-term development of this product there are only a few very short studies concerned with the nature of the phenomena linked to the elaboration process (*4*).

Owing to market demands, production of sherry vinegar today is performed by the majority of companies in a specific and controlled way, although this was not always the case in the past, when it was considered as a subproduct resulting from problems with the sherry-making process. Indeed, we could say that the nature of sherry vinegar evolved in tandem with the history of sherry production.

Initially, vinegar was simply the result of an alteration in the critical phase of the production process or an unsuccessful attempt to obtain a good sherry. For example, a batch of this product may for some reason have been left during long periods of aging, during which it underwent a complicated process of acetification conditioned by the high alcohol content and the complex nature of the aged sherry.

Nowadays, a high proportion of the raw material used for the production of sherry vinegar consists of perfect young sherry wine because accidental incidents in which the sherry wine is left for prolonged periods very rarely occur during the elaboration process. However, such incidents have not been completely eliminated and some of the faulty matured wines enter this system. For this reason some proportion of the sherry wine, in the first phases of maturing, is destined for the production of vinegar.

This situation significantly changes the production methods, because the acetification process is currently carried out using biological reactors and the product is later subjected to the traditional methods of aging (*5*). Therefore, the raw material

* To whom correspondence should be adressed. Fax: 00 34-956-016411. E-mail: victor.palacios@uca.es.
† University of Cadiz.
‡ Department of Research and Development, Pedro Domecq.

10.1021/jf020093z CCC: $22.00
Published on Web 06/14/2002

for sherry vinegar consists of wines of different natures with a high proportion of young wines, although these proportions partly depend on the specific character of the in-house production method.

The aim of the study described here was to evaluate the chemical and biochemical transformations taking place in sherry vinegar in the different aging stages. These transformations are compared to the evolution of sherry wine during the biological aging (fino or manzanilla sherry wine) or the physicochemical aging (oloroso sherry wine).

MATERIALS AND METHODS

Vinegar Samples. The samples used in this study were obtained from traditional "solera system" sherry vinegar. In the solera system the vinegar was stored in American oak butts (1 butt = 500 L) and it had the following aging stages: vintage (250 butts), a young vinegar and raw material for the solera system aging process; criadera 2nd (300 butts), the youngest aging stages in the solera system with an average age of 2 years; criadera 1st (300 butts), average age of 3.5 years; solera (300 butts), average age of 5 years; old solera (10 butts), average age of 50 years; and very old solera (10 butts), the oldest aging stages in the solera system with an average age of 75 years maturing.

A 50-mL sample was taken from each butt and aging stage of vinegar, except for old solera and very old solera, from which 100-mL samples were taken. The samples were arranged in sets according to the aging stages and were subsequently analyzed. Three samplings per year were carried out during the two years of this study, and the vinegar was always taken out from the solera system before the commercial vinegar blend was produced.

Physicochemical Analysis. Total acidity (*6*) was determined by volumetric neutralization with sodium hydroxide using phenolphthalein as an acid/base indicator. The alcohol grade (*6*) was determined by distillation of the vinegar. The alcohol obtained in the distillate was measured by digital densimetry with an Anton Paar densimeter (model DMA 46, Anton Paar GmbH, Graz, Austria). The density (*7*) of the liquid was determined by digital densimetry using the same Anton Paar densimeter as above. Fixed acidity (*6*) was determined by volumetric neutralization (with sodium hydroxide) of the non-volatile acids in the dry sediments of the vinegar using phenolphthalein as an acid/base indicator. The volatile acidity (*6*) was obtained by the difference between total and fixed acidity. The pH (*6*) was measured with a digital pH meter (Beckman 3500, Beckman Instruments, Fullerton, CA). The extract content (*6*) was determined by gravimetry, with the vinegar evaporated using a water bath and the sediments dried in a heater at 105 °C. The ash content (*6*) was also determined by gravimetry, with the vinegar evaporated using a water bath and the sediments burnt in an electric oven at 525 °C. Sulfate levels (*6*) were determined by gravimetry as follows: the sulfates in the vinegar were precipitated with barium hydroxide and the precipitate produced was separated by filtration. The total nitrogen content (*8*) was determined by the Kjeldahl method. Cations (iron, copper, zinc, calcium, and magnesium) (*9*) were determined by atomic absorption spectrophotometry using an atomic absorption spectrophotometer (Perkin-Elmer 460, Perkin-Elmer Corporation, Norwalk, CT). Cations (sodium and potassium) (*9*) were determined by flame emission spectrophotometry with a flame emission spectrophotometer (Perkin-Elmer 460). Color (absorbance at 470 nm) (*7*) was measured by UV−Visible spectrophotometry with an UV−Visible spectrophotometer (Perkin-Elmer 200). Amino acids (*10*) were determined by HPLC with the formation of phenylisothiocyanate derivatives (PITC) and subsequent detection of the derivatives (HPLC chromatograph equipped with two high-pressure pumps (1 mL/min), automatic injector (10 μL), furnace at 30 °C with a 30 cm Pico tag column (Waters, Milford, MA), and a diode array detector (254 nm) (Waters). Volatile compounds (acetaldehyde, ethyl acetate, methanol, 2-butanol, 1-propanol, 1-butanol, isobutanol and isoamyl alcohol) (*11*) were determined by gas chromatography with a Hewlett-Packard 5890 chromatograph equipped with a capillary injector (250 °C), flame ionization detector (250 °C), 50-m capillary column (HP−FFAP from Hewlett-Packard) (45−200 °C, with 3 °C/min ramp), split injection with a 1:50 ratio and nitrogen as the carrier gas (Hewlett-Packard, Palo Alto, CA). Organic acids (citric, tartaric, gluconic, malic, succinic, and lactic) and glycerol (*12*) were determined by HPLC with a Waters chromatograph equipped with a high-pressure pump (1.2 mL/min), automatic injector (7 μL), furnace at 55 °C, a 60-cm Fast-Fruit-Juice column from Waters and two detectors: a diode array detector (210 nm) was used for citric, tartaric, gluconic, and malic acids and a refractive index detector was used for succinic acid, lactic acid, and glycerol (Waters). The mobile phase was phosphoric acid at 0.025% in Milli-Q quality purified water (Millipore, Bedford, MA).

RESULTS AND DISCUSSION

Evolution of the different parameters during the sherry vinegar elaboration process can be divided into two well-defined periods during which two characteristic phenomena are evident. First, a biological process develops during the first phase (from the initial vintage stage to the solera stage) and this is characterized by the fermentation activity of the acetic acid bacteria from genus *Acetobacter* and *Gluconobacter* at the liquid surface (*13*). This natural stage, despite its different behavior, appears similar to that developed by the yeast during the biological aging beneath the yeast film of fino and manzanilla type sherries. Secondly, a physicochemical aging phenomenon develops at a later stage, without significant biological activity, during the prolonged aging phase. This second phase encompasses the solera vinegar and very old solera vinegar stages. In this latter period, physicochemical aging phenomena are observed in certain sherries including oloroso or amontillado. Likewise, during elaboration the sherry vinegar acquires a particular composition after a number of years, essentially due to the two aforementioned phenomena and to the particular raw material used, i.e. several types of sherries that have to some extent experienced a certain degree of an intense biological maturing process.

Biological Activity Phase. Variation of the general parameters during the maturing of sherry vinegar is shown in **Table 1**. As can be seen, during the biological activity phase the total acidity increases progressively, mainly in the first aging stages. This increase in acidity is due to the well-known fermentation activity caused by the acetic acid bacteria in the film culture (*13*). However, the conversion rate of ethanol into acetic acid is very slow when compared to the corresponding fermentation in the submerged culture (*13*). The slow bioconversion rate is due to the reduced surface area of the bacterium in relation to that of the substrate.

During this first aging phase, as a consequence of the acetic bacteria metabolism, an increase in the quantities of some acids is produced, including gluconic, succinic, and citric acids. On the other hand, a decrease in other acids is observed, and these include lactic acid and malic acid, which are oxidized to CO_2 and H_2O (*9*). These data have been confirmed in other acetification studies (*14*, *15*).

Furthermore, tartaric acid, potassium, and calcium contents increase by virtue of dissolution of the encrusted salts in the used barrels, which have usually contained sherry wine in the past (**Table 1**). Such dissolution processes are favored by the continuous decrease in pH that occurs during this stage (**Table 1**). Glycerol is an important component of sherry and is produced during the fermentation of the alcohol. Many authors have confirmed that glycerol is oxidized by the acetic bacteria during the acetic acid fermentation process (*15*−*17*); however, our studies reveal that, in the case of film growth, the acetic bacteria do not consume glycerol during the biological activity phase (**Table 1**). Therefore, the values observed for this vinegar are higher than those found in other aged vinegars (4.5 g/L)

Table 1. Analysis of the Sherry Vinegars Studied in Their Different Stages of Aging (means ($n = 6$) ± standard deviation)

	stage of aging					
	biological phase				physicochemical phase	
	vintage	criadera 2nd	criadera 1st	solera	old solera	very old solera
average age (years)	0	2	3.5	5	50	75
total acidity[a] (% w/v)	1.02 ± 0.61	7.08 ± 1.15	9.96 ± 0.32	10.38 ± 0.21	11.8 ± 0.43	11.4 ± 0.7
alcohol grade[b] (% v/v)	13.6 ± 0.9	4.0 ± 1.2	0.9 ± 0.3	0.5 ± 0.1	1.6 ± 0.1	1.5 ± 0.1
density[b] (g/mL)	0.9900 ± 0.006	1.013 ± 0.003	1.024 ± 0.004	1.026 ± 0.004	1.050 ± 0.005	1.063 ± 0.005
volatile acidity[a] (g acetic ac/L)	6.6 ± 5.3	66.9 ± 10.4	94.8 ± 3.8	98.5 ± 1.9	111.7 ± 3.7	106.3 ± 3.3
pH[c]	3.03 ± 0.10	2.72 ± 0.06	2.62 ± 0.05	2.6 ± 0.05	2.85 ± 0.04	2.9 ± 0.02
acetic acid[a] (% w/v)	0.66 ± 0.53	6.69 ± 1.04	9.48 ± 0.38	9.85 ± 0.19	11.17 ± 0.37	10.63 ± 0.33
tartaric acid[d] (g/L)	2.82 ± 0.24	3.32 ± 0.31	3.67 ± 0.33	3.85 ± 0.41	2.29 ± 0.17	1.85 ± 0.15
citric acid[d] (g/L)	0.35 ± 0.15	0.46 ± 0.19	0.66 ± 0.20	0.74 ± 0.23	1.95 ± 0.41	0.24 ± 0.06
gluconic acid[d] (g/L)	0.15 ± 0.10	0.51 ± 0.17	0.72 ± 0.22	1.17 ± 0.31	0.81 ± 0.19	1.23 ± 0.21
malic acid[d] (g/L)	0.1 ± 0.08	0.06 ± 0.05	0.05 ± 0.05	0.01 ± 0.01	0.16 ± 0.04	0.34 ± 0.11
succinic acid[d] (g/L)	0.27 ± 0.14	0.26 ± 0.16	0.42 ± 0.24	0.56 ± 0.21	2.28 ± 0.47	3.32 ± 0.54
lactic acid[d] (g/L)	0.17 ± 0.13	0.11 ± 0.07	0.06 ± 0.04	0.03 ± 0.02	2.00 ± 0.11	3.47 ± 0.17
dry extract[e] (g/L)	14.4 ± 2.3	18.5 ± 1.9	21.1 ± 1.7	25.2 ± 2.3	80.6 ± 4.7	128.8 ± 7.1
glycerol[d] (g/L)	3.2 ± 1.8	4.8 ± 0.9	5.3 ± 0.7	5.9 ± 0.6	15.3 ± 1.2	21.6 ± 0.9
ash[e] (g/L)	3.2 ± 0.3	2.5 ± 0.3	3.3 ± 0.4	3.8 ± 0.4	12.8 ± 0.7	18.1 ± 0.8
sulfates[e] (g K_2SO_4/L)	1.88 ± 0.25	2.23 ± 0.31	2.68 ± 0.30	2.52 ± 0.26	7.81 ± 0.51	7.21 ± 0.53
iron[f] (mg/L)	11.5 ± 3.4	12.2 ± 2.8	9.0 ± 2.2	18.0 ± 3.1	38.0 ± 5.8	48.2 ± 4.7
copper[f] (mg/L)	1.31 ± 0.49	0.76 ± 0.24	0.70 ± 0.20	0.90 ± 0.18	1.85 ± 0.17	2.41 ± 0.15
sodium[g] (mg/L)	59 ± 12	46 ± 10	39 ± 8	54 ± 7	420 ± 19	510 ± 22
potassium[g] (mg/L)	905 ± 55	1148 ± 70	1210 ± 67	1272 ± 73	5155 ± 120	7426 ± 218
calcium[f] (mg/L)	130 ± 10	170 ± 14	210 ± 17	190 ± 16	235 ± 17	250 ± 18
magnesium[f] (mg/L)	75 ± 5	89 ± 7	101 ± 7	109 ± 8	430 ± 19	760 ± 23
zinc[f] (mg/L)	1.4 ± 0.2	0.7 ± 0.1	0.9 ± 0.2	1.0 ± 0.2	6.5 ± 0.3	6.6 ± 0.3
absorbance 470 nm[h] (au)	0.095 ± 0.030	0.207 ± 0.045	0.367 ± 0.052	0.494 ± 0.061	3.770 ± 0.125	6.260 ± 0.218

[a−h] Methods for determining each compound: [a] titration, [b] densimetry, [c] pH meter, [d] HPLC [e]gravimetry, [f] atomic adsorption spectrophotometry, [g] atomic emission spectrophotometry, and [h] UV–Visible spectrophotometry.

(*3*). Consequently, the glycerol values in sherry vinegars will greatly depend on the original raw material and, to a greater degree, on the extent of the biological aging that the sherry wines have undergone, as during this period the yeast film consumes a significant amount of glycerol (*19*−*21*).

The reduction of Cu^{2+} and Zn^{2+} is detected during this phase of biological activity involving the acetic bacteria, possibly due to consumption by the acetic bacteria of the prosthetic groups of the enzymes and later by precipitation with the proteins (**Table 1**).

Finally, there is an increase in the sample absorbance at 470 nm during this phase, mainly due to the increase in the concentration of polyphenol compounds and to an increase in the oxidation, polymerization, and condensation of substances leaching out of the oak butts (*21*, *22*) (**Table 1**).

Physicochemical Activity Phase. When analyzing the variation between the solera vinegar and the very old solera vinegar, a behavior is observed that is different from that produced during the biological activity phase. For example, the total acidity becomes stable, which shows that the fermentative activity of the acetic bacteria has practically ceased in the medium and given way to physicochemical phenomena.

One of these phenomena is a significant loss of water by evaporation. It has been demonstrated (*23*) that the vegetable fibers of the oak wood in the butts will allow evaporation of water molecules through the wood but will prevent the passage of organic molecules. The vinegar lost as a result of this effect in the aging can be estimated to be 3−5% of the initial volume each year (*23*), and many substances will increase in concentration accordingly. This concentration phenomenon has also been observed during the aging of oloroso and amontillado sherry wines (*21*, *24*). Such a loss of water produces a considerable increase in the dry extract levels and density of the medium during this period (**Table 1**). It is, therefore, expected that there will be an increase in the concentration of the more stable components from a physicochemical point of view (e.g., succinic, malic, or lactic acids, plus the majority of cations, such as sodium, iron, copper, zinc, calcium, magnesium, and potassium). Potassium in particular experiences an increase of more than 700% due to dissolution from the oak wood of the barrels.

On the other hand, the loss of water during this phase produces a decrease (by precipitation) in the concentration of other substances, such as tartaric acid (**Table 1**), as a result of the increase in the solubility product of the salt. As a consequence, tartaric acid is consumed and potassium bitartrate is precipitated, thus producing an increase in the pH of the sample.

Moreover, an increase in absorbance can be observed because of the increase in the concentration of polyphenol compounds and an increase in the oxidation of these substances. The increase in absorbance could also be due to Maillard reactions, resulting from the combination of amino acids with molecules that contain carbonyl groups (**Tables 1** and **2**).

Now that the essential phases of the maturing stage of the sherry vinegar have been analyzed, the evolution of some substances of special interest will be discussed in the following sections.

General Variation of Amino Acids. It is generally observed that the content in total amino acids increases throughout the biological activity phase (**Table 2**) and, in fact, doubles in concentration from the vintage stage to the solera stage. This increase is a direct consequence of the bacteria metabolism and lysis. The most significant increase in amino acid levels (80%) coincides with the time of the highest fermentation activity, which is produced at year two of the process. This effect is opposite of that observed in the biological aging of sherry wine, where a significant consumption of amino acids occurs (*20*, *25*).

The most marked changes in the amino acids under study occur for proline and arginine, with increases of 231 and 510 mg/L, respectively, during this first phase (**Table 2**). Only small

Table 2. Variation of Total Nitrogen and of the Different Amino Acids during the Aging Process of Sherry Vinegars[a]

	stage of aging					
	biological phase				physicochemical phase	
	vintage	criadera 2nd	criadera 1st	solera	old solera	very old solera
average age (years)	0	2	3.5	5	50	75
total nitrogen[b] (mg/L)	274 ± 38	431± 51	492 ± 47	535 ± 62	688 ± 38	636 ± 43
phosphoserine (mg/L)	1.1 ± 0.8	4.7 ± 0.9	5.7 ± 1.2	7.2 ± 0.9	9.5 ± 0.5	16.1 ± 0.7
aspartate (mg/L)	8.9 ± 1.0	12.7 ± 1.3	17.2 ± 1.3	24.8 ± 1.2	8.6 ± 0.9	14.7 ± 0.5
glutamate (mg/L)	24.9 ± 2.5	31.1 ± 2.7	34.9 ± 2.3	40.1 ± 3.2	11.9 ± 0.6	22.1 ± 1.1
serine (mg/L)	6.6 ± 1.1	10.0 ± 1.5	14.5 ± 1.3	19.3 ± 1.0	5.2 ± 0.9	6.1 ± 1.2
asparagine (mg/L)	26.4 ± 3.6	35.7 ± 1.2	41.1 ± 1.6	45.8 ± 0.8	2.3 ± 0.2	5.1 ± 0.3
glycine (mg/L)	8.3 ± 0.9	15.2 ± 1.0	21.3 ± 0.9	30.1 ± 1.2	39.3 ± 1.6	86.6 ± 2.3
histidine (mg/L)	6.3 ± 0.8	13.3 ± 1.3	15.5 ± 1.5	16.6 ± 2.0	3.6 ± 0.9	5.5 ± 0.5
γ-aminobutyric (mg/L)	9.0 ± 1.7	20.9 ± 2.6	21.8 ± 3.4	26.9 ± 2.9	5.2 ± 1.6	11.9 ± 1.6
threonine (mg/L)	5.4 ± 0.9	10.9 ± 1.1	15.4 ± 2.0	19.2 ± 2.3	4.5 ± 0.6	4.0 ± 0.7
alanine (mg/L)	17.8 ± 1.6	33.9 ± 2.5	38.0 ± 1.5	44.9 ± 1.0	28.1 ± 1.6	52.7 ± 1.1
arginine (mg/L)	33.7 ± 5.9	288.4 ± 19.1	263.0 ± 18.0	231.5 ± 14.5	17.4 ± 1.9	19.4 ± 1.3
proline (mg/L)	371.2 ± 21.3	494.2 ± 39.0	516.4 ± 35.2	509.9 ± 21.5	504.1 ± 14.2	497.1 ± 13.9
α-aminobutyric (mg/L)	1.6 ± 0.2	0.9 ± 0.2	1.0 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	0.9 ± 0.1
tyrosine (mg/L)	8.6 ± 0.8	17.0 ± 1.2	23.3 ± 2.3	27.1 ± 1.6	11.0 ± 1.9	10.1 ± 1.5
valine (mg/L)	5.3 ± 1.9	11.9 ± 1.3	18.2 ± 2.0	24.8 ± 2.1	17.4 ± 1.8	25.4 ± 2.5
methionine (mg/L)	2.6 ± 1.3	4.9 ± 1.6	6.2 ± 1.6	6.8 ± 1.9	0.8 ± 0.3	1.0 ± 0.2
cysteine (mg/L)	16.5 ± 3.5	16.2 ± 2.9	10.1 ± 1.8	9.4 ± 2.1	9.0 ± 1.7	10.5 ± 0.9
isoleucine (mg/L)	3.2 ± 1.2	6.5 ± 1.4	11.1 ± 1.6	15.5 ± 1.3	14.6 ± 1.1	19.5 ± 1.0
leucine (mg/L)	12.1 ± 3.2	20.1 ± 2.9	31.3 ± 2.3	40.6 ± 3.7	16.3 ± 2.8	22.0 ± 1.9
phenylalanine (mg/L)	10.0 ± 4.6	17.3 ± 2.5	25.4 ± 4.9	32.6 ± 2.9	12.2 ± 1.9	11.8 ± 0.9
tryptophan (mg/L)	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ornithine (micromol/L)	5.3 ± 2.3	20.8 ± 2.6	19.7 ± 2.7	17.3 ± 1.8	1.5 ± 0.3	3.4 ± 0.5
lysine (mg/L)	14.6 ± 3.4	30.0 ± 5.9	38.6± 2.1	43.2 ± 2.7	5.5 ± 0.6	11.0 ± 1.0
total amino acids[c] (mg/L)	600 ± 63.7	1118 ± 96.7	1191 ± 91.6	1235 ± 73	730 ± 38	858 ± 35

[a] Means (n=6) ± standard deviation. [b] Total nitrogen was determined by Kjeldhal. [c] Amino acids were determined by HPLC.

Table 3. Variation of Volatiles Compounds during the Aging Process of Sherry Vinegars[a,b]

	stage of aging					
	biological phase				physicochemical phase	
	vintage	criadera 2nd	criadera 1st	solera	old solera	very old solera
average age (years)	0	2	3.5	5	50	75
acetaldehyde (mg/L)	75 ± 61	65 ± 31	37 ± 16	22 ± 14	105 ± 21	92 ± 16
ethyl acetate (mg/L)	865 ± 415	2246 ± 518	613 ± 304	206 ± 101	1081 ± 315	582 ± 179
methanol (mg/L)	67 ± 37	50 ± 24	35 ± 17	21 ± 9	34 ± 7	34 ± 9
2-butanol (mg/L)	ND[c]	ND	ND	ND	ND	ND
n-propanol (mg/L)	62 ± 12	15 ± 4	ND	ND	4 ± 2	8 ± 3
i-butanol (mg/L)	52 ± 16	21 ± 7	16 ± 4	ND	13 ± 6	14 ± 7
n-butanol (mg/L)	ND	ND	ND	ND	ND	ND
i-amyl alcohols (mg/L)	151 ± 75	46 ± 37	20 ± 11	ND	19 ± 12	18 ± 10
acetoine (mg/L)	150 ± 21	512 ± 39	571 ± 42	595 ± 45	650 ± 67	680 ± 71

[a] Means (n=6) ± standard deviation. [b] Volatile compounds were determined by GC. [c] ND, not detected.

increases in concentration are produced for the rest of the amino acids. Traditionally, the richness in amino acids of sherry vinegar has been considered a genuine element of its wine origin and, in fact, the Spanish regulations do not refer to the maximum content of proline in vinegars. However, the regulations consider that contents lower than 80 mg/L correspond to vinegars obtained from sources other than wine or that the vinegar has been produced with synthetic acetic acid (*26*).

On the other hand, a decrease in the total amino acid concentration is observed during the second phase of physicochemical maturing (**Table 2**), especially in the initial stages. Later, the values are practically constant from the solera to the very old solera stages or, in some instances, they increase slightly. This initial global decrease, which is contrary to the observed concentration effect, is mainly due to the fact that amino acids are involved in different reactions (mainly amino−carbonyl) that finally result in the formation of Maillard compounds. These nonenzymatic reactions result in browning of the product and are activated by the presence of certain concentrations of heavy metals (e.g., Fe). Such reactions are in some way responsible for the marked increase in absorbance produced in the vinegar during the physicochemical phase (**Table 2**) and contribute to an increase in the color and aroma of the product.

This behavior is not observed for glycine and alanine, which both increase in concentration during this period. The presence of these two compounds can increase from phenomena associated with oak wood hydrolysis and dissolution from the walls of butts (**Table 2**).

Finally, it can be seen for very old vinegars that there is a balance between the water loss phenomenon, which tends to increase the concentration of amino acids, and the Maillard reactions, which lead to a decrease in the concentrations of amino acids.

General Variation of Volatile Compounds. In this context the variations in ethyl acetate during the maturing process are worthy of special mention (**Table 3**). The concentration of ethyl acetate depends, almost exclusively, (according to the law of

mass action) on the alcohol content and on the acidity of the vinegar; between which there is a high degree of correlation. Therefore, in very old vinegars that have significant amounts of residual alcohol (>1% ethanol v/v), it is possible to find high levels of ethyl acetate (between 0.5 and 1.0 g/L) in comparison to other vinegars (*2*).

Acetaldehyde, the intermediate metabolite of acetic fermentation, is not accumulated during the biological activity phase but decreases because of the oxidative fermentation metabolism.

Likewise, a general decrease in the higher alcohols can be observed during the solera aging phase. Moreover, the concentrations of *n*-propanol, isobutanol, and isoamylic alcohols decrease to undetectable levels in this phase. This decrease can be attributed to the general aerobic metabolism in the bacteria from alcohols to acids and also to the formation of esters with acetic acid to give the corresponding acetates (**Table 3**).

The acetoin concentration increases significantly during the biological and physicochemical phases. In the first place, the initial concentration in the starting sherry wine is relatively high in comparison to that of other wines, in which the average concentration is between 7 and 15 mg/L (*24*). As mentioned previously, the reason for this situation is that sherry wine has undergone biological aging where yeast produces, by means of aerobic metabolism, a significant amount of acetoin to reach levels of 200 mg/L (*24*). In this way, and due to the reducing metabolism of yeast, butyleneglycol is also formed in the sherry wine (*24*) and this compound contributes to the formation of acetoin during the acetic fermentation (*2*).

Finally, the general water loss produced during the physicochemical activity gives way to a new increase in the acetoin concentration, where values of 680 mg/L can be found in the very old solera stage of the sherry vinegar.

LITERATURE CITED

(1) De Ley, J.; Schell, J. Oxidation of several substrates of *Acetobacter aceti*. *J. Bacteriol.* **1959**, *77*, 445−451.

(2) Troncoso, A.; Guzmán Chozas, M. Volatile components in Andalusian vinegars. *Z. Lebensm.-Unters. Forsch.* **1987**, *185*, 130−133.

(3) Troncoso, A.; Guzmán Chozas, M. Determination of glycerol in Andalusian vinegars. *Belg. J. Food Chem. Biotechnol.* **1988**, *43*, 112−114.

(4) Quirós, J. M. La elaboración de vinagre de calidad de jerez. *Quad. Scuola Spec. Vitic. Enol.* **1990**, *2*, 115−123.

(5) Dauberte, B.; Arzouyan, C.; Estienne, J. Selection des principaux critères analytiques des vinaigres par l'analyse de données. *Ann. Falsif. Expert. Chim.* **1993**, *86*, 55−68.

(6) *Métodos Oficiales de Análisis, Productos Derivados de la Uva (Sección Vinagre)*. Ministerio de Agricultura: Madrid, Spain, 1976; pp 56−68.

(7) O. I. V. *Recueil des methodes internationales d'analyse des vins*. Office International de la Vigne et du Vin: Paris, France, 1973; pp 27−30.

(8) Ribéreau-Gayon, J.; Peynaud, E.; Sudraud, P.; Ribéreau-Gayon, P. *Sciences et techniques du vin. Analyse et controle des vins*. Dunod: Paris, 1976; pp 390−399.

(9) *Métodos de Análisis Comunitarios Aplicables en el Sector del Vino*. A. Madrid Vicente Ediciones: Madrid, Spain, 1991; pp 114−213.

(10) Sanders, E. M.; Ought, C. S. Determination of free amino acids in wine by HPLC. *Am. J. Enol. Vitic.* **1985**, *36*, 43−46.

(11) Amerine, M. A.; Ought, C. S. *Methods for Analysis of Must and Wines*; John Wiley & Sons: New York, 1980; pp 103−127.

(12) Valcárcel, M. J.; González, P.; Pérez, L.; Asencio, A.; Domecq, B. Control de calidad del estado sanitario de la uva en la zona del jerez. *Vitivinicultura* **1990**, *5*, 42−49.

(13) Caro, I.; Palacios, V. M.; Perez, L. Kinetic models for the acetic acid fermentation. *Recent Res. Devl. Biotechnol. Bioeng.* **1998**, 203−211.

(14) Eschenbruch, B.; Dittrich, H. H. Metabolism of acetic acid bacteria in relation to their importance to wine quality. *Zentrabl. Microbiol.* **1986**, *141*, 279−289.

(15) Joeux, A.; Lafon-Lafourcade, S.; Ribereau-Gayon, P. Evolution of acetic acid bacteria during fermentation and storage of wine. *Appl. Environ. Microbiol.* **1984**, *48*, 153−156.

(16) Bousfield, E. G.; Wright, G. H.; Walker, T. K. Oxidation of glycerol by *Acetobacter* species. *J. Inst. Brew.* **1947**, *53*, 258−262.

(17) Stouthamer, A. H. Oxidative possibilities in the catalase positive *Acetobacter* species. *Antonie Van Leeuwenhoek J. Microbiol.* **1959**, *25*, 241−264.

(18) Hadorn, H.; Beetschem, W. Uberechte Gärungsessige mit extrem niedrigen Acetoingehalten. *Mitt. Geb. Lebensmittelunters. Hyg.* **1965**, *56*, 46−62.

(19) Casas, J. F. *Descripción resumida de la técnica enológica de los vinos de Jerez. III Jornadas Universitarias sobre el Jerez*. Servicio de Publicaciones, Universidad de Cádiz: Cádiz, Spain, 1985; pp 333−361.

(20) Martínez, P.; Valcárcel, M. J.; González, P.; Benítez, T.; Pérez, L. Consumo de etanol, glicerina y aminoácidos totales en vinos finos durante la crianza biológica bajo "velo de flor". *Aliment. Equipos. Tecnol.* **1993**, *12*, 61−65.

(21) Martinez de la Ossa, E.; Caro, I.; Bonat, M.; Pérez, L.; Domecq, B. Dry extract in sherry and its evolution in the aging process. *Am. J. Enol. Vitic.* **1987**, *38*, 321−325.

(22) Palacios, V. M.; Caro, I.; Pérez, L. Factors influencing the oxidation phenomena of sherry wine. *Am. J. Enol. Vitic.* **2001**, *52*, 151−155.

(23) Pérez, L. *Consideraciones técnicas en la elaboración del Jerez. II Jornadas Universitarias sobre el Jerez.* Servicio de Publicaciones, Universidad de Cádiz: Cádiz, Spain, 1982; pp 167−197.

(24) Martinez de la Ossa, E.; Perez, L.; Caro, I. Variations of the major volatiles through aging of sherry. *Am. J. Enol. Vitic.* **1987**, *38*, 293−297.

(25) Botella, M. A.; Pérez, L.; Domecq, B.; Valpuesta, V. Amino acids content of fino and oloroso sherry wines. *Am. J. Enol. Vitic.* **1990**, *41*, 12−15.

(26) Polo, M. C.; Suarez, M. A.; LLaguno, C. Aportación al estudio de los vinagres Españoles I. Contenido en aminoácidos libres y nitrógeno total. *A. T. A.* **1976**, *16*, 257−263.

Received for review January 28, 2002. Revised manuscript received May 1, 2002. Accepted May 3, 2002.

JF020093Z