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Changes in RNA Catabolites of Sparkling Wines During the Biological Aging

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ABSTRACT: In this study the catabolites derived from RNA degradation were assessed in Cava sparkling wines as a consequence of lees autolysis. For this purpose, the changes in the content of adenosine, guanosine, inosine, uridine, hypoxanthine, xanthine, and uric acid were determined by UHPLC-MS/MS, in sparkling wines produced on industrial scale, and aged for 4 years. Uridine is the main nucleoside, and its content increases whenever lees cells are present (*sur lie* aging). Purines seem to have a fermentative origin, with xanthine the most abundant one. When RNA catabolite amounts in sparkling wines aged with or without lees are compared over time, it can be concluded that lees and their cell degradation play an important role in the evolution of Cava; when lees are removed, RNA catabolite amounts remain unchanged.

KEYWORDS: *purines, uridine, nucleosides, autolysis, sparkling wine*

INTRODUCTION

The distinctive character of Cava (quality sparkling wine) is determined by the second fermentation and aging of the wine with yeast.[1] Once the second fermentation is complete, cell viability decreases by more than 90%,[2] autolysis starts, and biological aging takes place for a minimum of 9 months.[3] The contact between wine and lees, involving the reduction or increase of some components in wine, improves quality. Therefore, there is great interest among sparkling wine producers to know all the biochemical details of this biological aging. The elaboration of high-quality sparkling wines is a very long process, usually lasting between 18 and up to 30 months, and producers need information to control or monitor the procedure and justify the long aging and increased cost. The implications of the process called autolysis for sparkling wine have been cited previously.[4−8] During aging *sur lie*, some intracellular components are released from yeast cells, such as nitrogen compounds, peptides, polysaccharides, lipids, and nucleotides, which modify wine composition and final quality.[9] However, nucleic acids and their degradation products are among the least studied topics in sparkling wines. RNA and DNA make up 5−15% and 0.1−1.5% of cell dry yeast, respectively.[6] RNA, the main content of nucleic acids of yeast cells (95%), is degraded more rapidly than DNA.[10] Zhao and Fleet[11,12] concluded that up to 55% of total cell DNA and up to 95% of cell RNA were degraded during induced autolysis at 30−60 °C (pH 7.0), and at 40 °C (pH 4.0−7.0) for 10−14 days. Those authors suggested that RNA degradation is a key reaction of yeast autolysis. However, when autolysis was induced at 30 °C and pH 3 in hydroalcoholic buffer (ethanol 11.5%, v/v), only ribonucleotides were detected in yeast autolysate, as in up to 9 year-old Champagne. The absence of deoxyribonucleotides could be explained by a slower degradation of DNA, producing mainly oligonucleotides.[13] The concentration of nucleotides found in Champagne is very low, reaching a maximum of ∼3 mg L^{-1} for 9 years of aging.[13] However, in sparkling wine, the subsequent degradation of these nucleotides into nucleosides and purine bases has not yet been investigated in real oenological conditions mainly due to the reasons associated with analytical procedures. Recently, the development of soft ionization techniques such as electrospray ionization (ESI)[10] and MS/MS methods[14,15] facilitates the detection of these compounds in food. The ultrastructure of cell yeast is also clarified by the development of microscopic techniques, such as transmission electron microscopy (TEM).[16] The availability of these new analytical techniques permits the measurement of catabolic products that could be related to their autolytic status.

The aim of the study was to assess the level of seven RNA catabolites (guanosine, adenosine, inosine, uridine, xanthine, hypoxanthine, and uric acid) that can be found in Cava wines and to provide a better biochemical approach to understanding the real aging process, according to the yeast cell degradation evaluated by transmission electron microscopy (TEM). For this purpose an UHPLC−MS/MS method was used to determine changes in the concentration of seven target nucleosides and purines in three series of Cava sparkling wines produced on an industrial scale, and aged for 4 years in bottles with lees. Furthermore, to establish whether these target nucleosides and purines come from lees or from another biochemical mechanism, their level was also evaluated in two series of the same sparkling wines aged without lees contact.

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Table 1. Evolution of Catabolite Content (mg/L) of Cava Wine During Aging *"sur Lie"* (Different Letters Indicate Statistical Differences by ANOVA)

series	0 month	1 month	3 months	6 months	9 months	13 months	17 months	21 months	30 months	39 months	48 months
						Adenosine					
A	<0.01a[a]	<0.01a	<0.02a[b]	0.17h ± 0.01	0.09e ± 0.01	0.15f ± 0.01	0.16g ± 0.01	0.05c ± 0.01	0.07d ± 0.01		0.03b ± 0.01
B	<0.01a	<0.01a	<0 02b	0.07d ± 0.01	0.07d ± 0.01	0.09e ± 0.01	0.12f ± 0.01	0.07d ± 0.01	0.07d ± 0.01		0.04c ± 0.01
C	<0.01a	<0.01a	<0.01a	0.02b ± 0.01	0.18f ± 0.01	0.09d ± 0.01	*c*	0.14e ± 0.01		0.06c ± 0.01	
						Guanosine					
A	<0.06a[a]	<0.06a	<0.06a	0.46e ± 0.04	0.27c ± 0.03	0.27c ± 0.03	0.36d ± 0.03	0.24b ± 0.02	<0.06a		<0.06a
B	<0.06a	<0.06a	<0.06a	<0.06a	<0.06a	<0.18b[b]	<0.06a	<0.06a	<0.06a		<0.06a
C	<0.06a	<0.06a	<0.06a	<0.06a	<0.06a	0.20c ± 0.01		<0.18b		<0.06a	
						Uridine					
A	18.96ab ± 0.51	20.36bc ± 0.55	17.72a ± 0.48	20.87c ± 0.56	21.74c ± 0.59	20.37bc ± 0.55	25.12d ± 0.68	24.92d ± 0.67	26.62de ± 0.72		28.38e ± 0.77
B	10.81a ± 0.29	10.51a ± 0.28	13.31b ± 0.36	13.50b ± 0.36	13.45b ± 0.36	14.21bc ± 0.38	15.53de ± 0.42	14.73cd ± 0.40	15.96e ± 0.43		18.03f ± 0.49
C	15.46a ± 0.42	16.17a ± 0.44	16.68a ± 0.45	18.39b ± 0.50	16.11a ± 0.43	19.33b ± 0.52		19.11b ± 0.52		22.10c ± 0.60	
						Hipoxanthine					
A	<0.44a[b]	<0.44a	0.64c ± 0.06	1.03e ± 0.10	0.99e ± 0.09	0.82d ± 0.08	0.81d ± 0.08	0.53bc ± 0.05	0.45b ± 0.04		0.95e ± 0.09
B	0.54a ± 0.05	0.97b ± 0.09	1.28fg ± 0.12	1.00bc ± 0.09	1.14de ± 0.11	1.05bcd ± 0.10	1.19ef ± 0.11	1.43h ± 0.13	1.30g ± 0.12		1.07cd ± 0.10
C	<0.44a	1.70bc ± 0.16	1.80cd ± 0.17	1.74bcd ± 0.16	1.84cd ± 0.17	1.88d ± 0.18		1.61b ± 0.15		1.85d ± 0.17	
						Xanthine					
A	8.11a ± 0.26	9.86bc ± 0.32	9.38b ± 0.30	9.72bc ± 0.31	9.82bc ± 0.31	9.59bc ± 0.31	9.47bc ± 0.30	9.85bc ± 0.32	10.20c ± 0.33		10.08bc ± 0.32
B	10.19ab ± 0.33	10.29a ± 0.33	11.72cd ± 0.37	11.27bcd ± 0.36	11.31cd ± 0.36	11.25bcd ± 0.36	11,25bcd ± 0,36.1	1.07abcd ± 0.35	11.78d ± 0.38		10.80abc ± 0.35
C	9.54a ± 0.31	10.58bc ± 0.34	10.16abc ± 0.32	10.15abc ± 0.32	10.83c ± 0.35	10.70bc ± 0.34		9.93ab ± 0.32		10.88c ± 0.35	
						Uric Acid					
A	0.74a ± 0.07	0.77bc ± 0.08	1.17f ± 0.12	1.31g ± 0.13	1.02e ± 0.10	0.77bc ± 0.08	0.75b ± 0.07	0.93de ± 0.09	0.78bc ± 0.08		0.88cd ± 0.09
B	0.65a ± 0.06	0.92e ± 0.09	0.83d ± 0.08	0.64ab ± 0.06	0.73c ± 0.07	0.80d ± 0.08	0.70bc ± 0.07	0.84d ± 0.08	0.81d ± 0.08		0.82d ± 0.08
C	0.44c ± 0.04	0.40b ± 0.04	<0.36a[b]	0.51f ± 0.05	0.49dc ± 0.05	0.47cd ± 0.05		0.49de ± 0.05		0.45c ± 0.04	

[a]<LOD. [b]<LOQ. [c]Nonexistent sample point.

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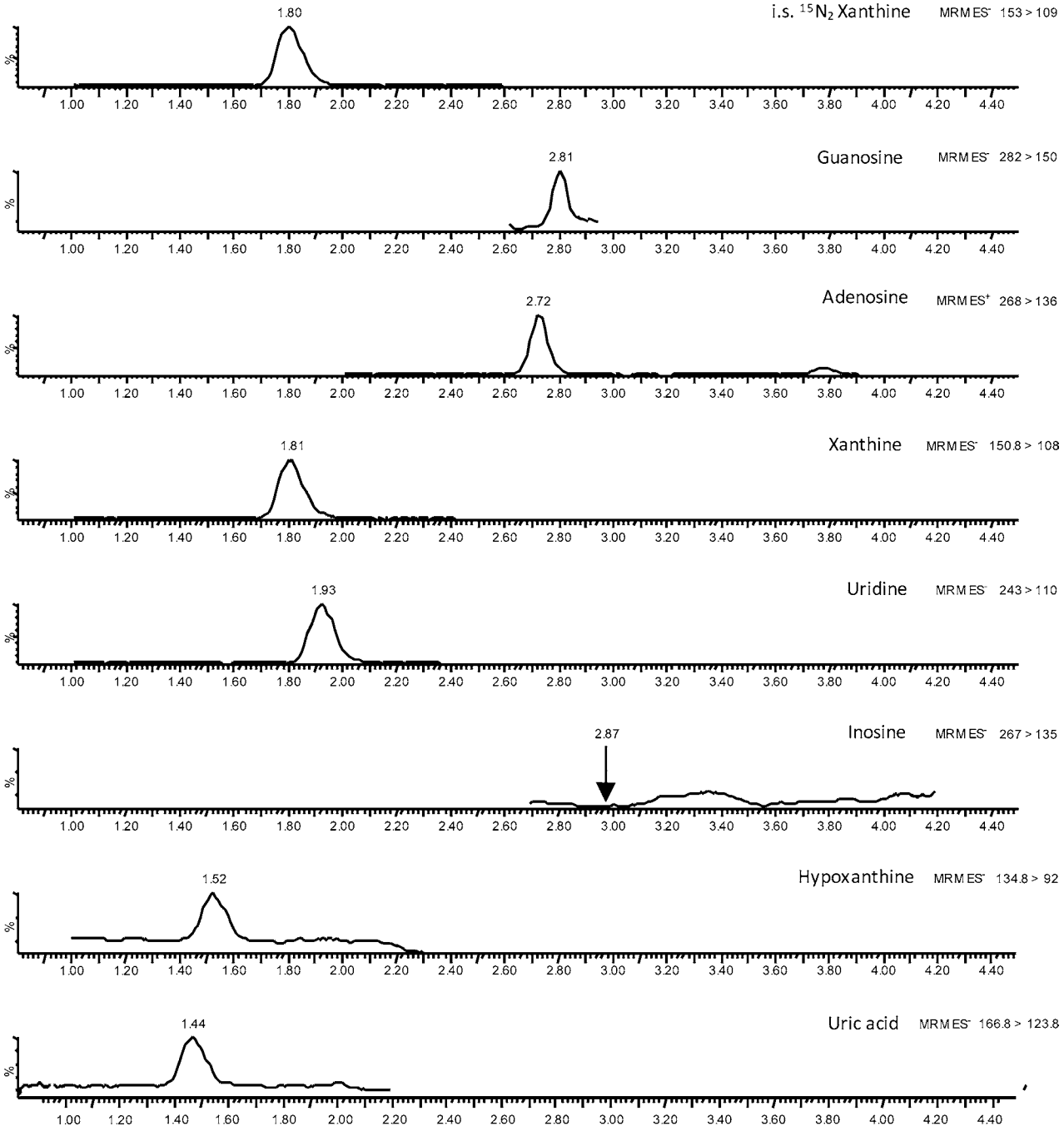

Figure 1. MRM profile corresponding to the quantifier transition of adenosine, guanosine, inosine, uridine, hypoxanthine, xanthine, and uric acid found in a Cava sample.

MATERIALS AND METHODS

Samples. Three Cava sparkling wine series (series A, B, and C) from three different blends were made following the traditional method on an industrial scale at the Freixenet S.A. winery. The three base wines were bottled after the addition of the same "liqueur de tirage". This liqueur was composed of 1−2 × 10^6 yeast cells mL^{-1} (yeast starter), 500 g L^{-1} of sucrose, and 0.04 g L^{-1} of calcium bentonite. As approximately 40−42 mL of "liqueur de tirage"/750 mL bottle was added, each bottle received between 22 and 24 g of sucrose for the second fermentation.[17] The *S. cerevisiae* F11 strain belongs to the private collection of Freixenet S.A. winery. Each Cava series was stacked in two cages of 500 bottles of 750 mL and stored (16 °C) in the cellar for 48 months during the second fermentation "*prise de mousse*" and aging in contact with lees. Two bottles of each series were taken at different aging times. These sampling points were selected according to representative aging periods: 0 months (base wine added of "*liqueur de tirage*"), 1 month (second fermentation), 3 months (end second fermentation), 6 months (aging), 9 and 13 months (Cava), 17 and 21 months (Reserva), 30, 39, and 48 months (Gran Reserva).

In addition, 500 bottles of Cava (one cage of each series A and B) were disgorged to remove the lees at 9 months of biological aging. The brut expedition liqueur (sucrose, wine, and sulfite) was added automatically to produce commercial sparkling wines. These two series of Cava without lees (named Ac and Bc) were stored together with A, B, and C cages. Subsequently, they are under the same cellar conditions at 16 °C for four years. At 13, 17, 21, 39, and 48 months of aging, coinciding with the sampling time for series A and B, two bottles were collected for series Ac and Bc.

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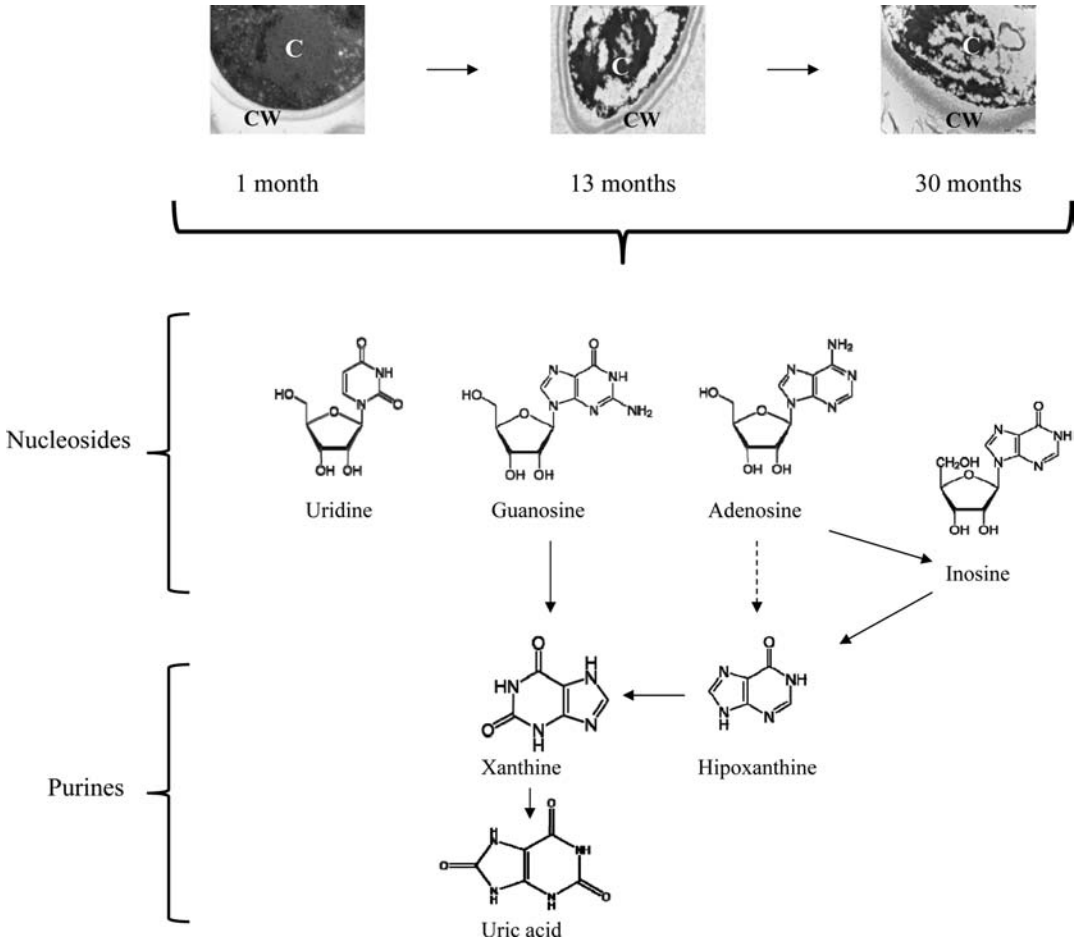

Figure 2. Catabolic pathways of nucleosides and purines in sparkling wines according to yeast cell degradation observed by TEM. CW = cell wall; C = cytoplasm.

In order to remove the lees and to degas the product, the contents of bottles from series A, B and C were centrifuged at 4 °C for 15 min at 1410*g* (Rotina48CR, Tuttlingen, Germany). The supernatant was then placed in a 250 mL amber flask. Aseptic conditions were maintained throughout the experiment. The samples from series Ac and Bc were also degassed by magnetic stirred under sterile conditions in the laminar flow hood.

All samples were then stored at −20 °C until analysis.

Chemical Standards and Reagents. Adenosine, guanosine, inosine, hypoxanthine, xanthine, uric acid, hydrochloric acid, and ammonium hydroxide ACS were supplied by Sigma-Aldrich (St. Louis, MO).Uridine and formic acid mass spectrometry grade were from Fluka (Buchs, Switzerland). Deuterated xanthine [1,3-^{15}N2] used as an internal standard with isotopic purity greater than 98%, was purchased from Cambridge Isotope Laboratories (Andover, MA). Acetonitrile LC-MS grade was from Riedel-de Haën (Seelze, Germany), while ultrapure water was obtained by a Milli-Q system (Millipore, Milford, MA). Single-standard stock solutions were prepared weekly by dissolving known amounts of pure standards in 2 mM ammonium hydroxide and then storing at 4 °C until analysis. Internal standard solution (I.S.) was prepared weekly by dissolving known amounts of 1,3-$^{15}N_2$-xanthine in 2 mM ammonium hydroxide, diluting with water to reach a final concentration of 20 μg mL^{-1}, and then storing at 4 °C.

UPLC–MS/MS analyses. Analyses were carried out following a previously published method[14] with an Acquity Ultra-Performance liquid chromatography system (UPLC) (Waters, Milford, MA), equipped with a binary pump system, a photodiode array detector (DAD), a ZSpray electrospray (ESI) as a source of ionization, and a triple-quadrupole mass spectrometer (TQD). Chromatographic separation was achieved with a BEH C_{18} Shield column (150 mm × 1.0 mm id) with 1.7 μm particle size (Waters, Milford, MA), kept at 40 °C. An isocratic elution was carried out with acetonitrile (ACN)/water/formic acid (5:94.9:0.1, v/v/v) with a flow rate of 0.140 mL min^{-1}. Sample vials and the injector were maintained at 4 °C; the injected volume was 2 μL. Electrospray was operated for all analytes in negative (ESI$^-$) mode, except for adenosine which was performed in positive (ESI$^+$) mode.[14] Data acquisition and integration were controlled by MassLynx software (version 4.0). Cava samples (500 μL) were added with 450 μL of formic acid 0.1% in water, and 50 μL of [1,3-$^{15}N_2$] xanthine solution (i.s. = 20 mg L^{-1}), then filtered with PTFE membrane filter (0.22 μm porosity), and injected. Standard addition calibration curves were created by spiking two Cava samples with seven spiking levels for each compound. Calibration curves were made by plotting the peak area ratio of each compound to the internal standard against the concentration ratio of the compounds to the internal standard, taking into account the initial concentrations in real samples. The limit of detection (LOD) was calculated on the basis of the calibration curve response for each kind of matrix at S/N = 3. Intraday repeatability was determined by analyzing two samples in triplicate each day.

Transmission Electron Microscopy (TEM) of Wine Lees. The lees of three bottles were prepared at each sampling point by centrifugation (previously described in Samples section), and the pellet was washed three times with 30 mL of NaCl 0.9% (Panreac Barcelona, Spain) according to Leroy et al.[18]

Lees pellets were selected under a stereomicroscope, transferred to 1.5 mm diameter and 200 μm deep planchettes, and immediately cryoimmobilized, using a Leica EMPact high-pressure freezer (Leica, Vienna, Austria), and then stored in liquid nitrogen until further use.[19] After rapid freezing, specimens were freeze-substituted in anhydrous

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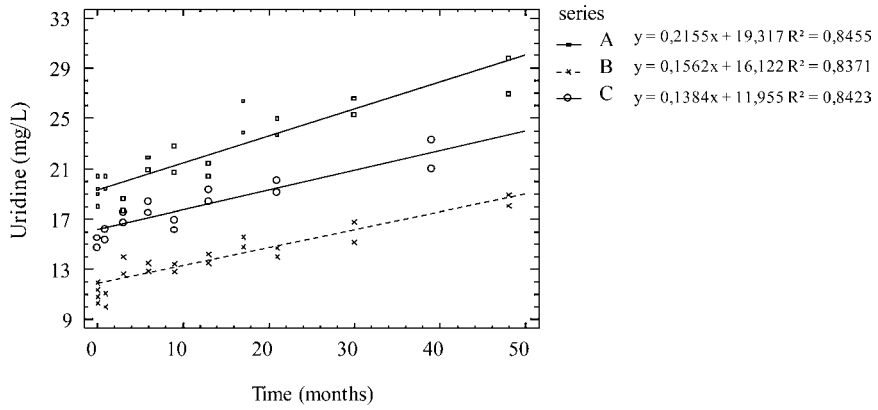

Figure 3. Uridine release in sparkling wine (Cava series A, B, and C) during aging *sur lie*.

acetone containing 2% osmium tetroxide and 0.1% uranyl acetate at −90 °C for 3 days. They were gradually warmed to room temperature at a temperature progression rate of 5 °C h.

After several acetone rinses, the samples were embedded in Epon resin. The Epon was then polymerized for 48 h at 60 °C.[20] Ultrathin sections were cut with a Leica Ultracut UCT ultra-microtome, mounted on Formvar-coated copper grids and poststained with 2% uranyl acetate in water and lead citrate.[21] The ultrathin sections were observed with a Tecnai Spirit electron microscope (FEI Co, Hillsboro, OR) at an accelerating voltage of 120 kV. On average, 10 overview and approximately 55−65 detailed electron micrographs for each sample point were taken.

Statistical Analysis. Statgraphics Plus 5.1 was used to carry out the statistical data analysis. Results were analyzed by simple and multifactorial ANOVA ($p < 0.05$). Relationships between catabolite content and aging time were also examined.

RESULTS AND DISCUSSION

The concentration of 4 nucleosides (guanosine, adenosine, uridine, and inosine) and 3 purine bases (hypoxanthine, xanthine, and uric acid) in the three different series (A, B, and C) of sparkling wine during their manufacturing process ("méthode champenoise") is shown in Table 1. The sampling points of this table were according to Cava categories: base wine (0 month), second fermentation (1 month), end of second fermentation, as indicated by Freixenet S.A data (3 months), post fermentative aging (6 months), "Cava" (9 and 13 months), "Reserva" (17 and 21 months), and "Gran Reserva" (30, 39, and 48 months).

In Figure 1 is reported the MRM profile corresponding to the quantifier transition of nucleosides and purine bases of Cava. Inosine was not detected in any sample analyzed of base wine or sparkling wine (Figure 1). This might be because inosine was below its detection limit (39 μg L^{-1}). However, another possibility is the enzymatic activity of purine-nucleoside phosphorylase (EC 2.4.2.1.) which catalyzes the direct transformation of inosine into hypoxanthine,[22] or from adenosine (Figure 2) by an intermediate step to adenine (EC 2.4.2.1.) and then throught adenine deamination (adenine deaminase EC 3.5.4.2.) to hypoxanthine (Figure 2 in discontinuous arrow).[23]

In base wine (Table 1, 0 months), uridine is the only nucleoside detected, whereas purines were detected in all series. Hypoxanthine shows values in the base wine lower than uric acid, whereas it remains in series A and C below the limit of quantification (44 μg L^{-1}).

In sparkling wine, all nucleosides (except the inosine mentioned above) and purines were quantified.

Levels of Nucleosides in Cava Wine During "sur Lie" Aging. Uridine was the main nucleoside, higher than adenosine and guanosine amounts (Table 1). The greater presence of uridine in the current study contradicts the previous research into model wines by Charpentier et al.[13] and Guilloux-Benatier and Chassagne.[9] These authors found that adenosine was the main nucleoside, followed by uridine and guanosine, when induced autolysis is performed. Nevertheless, our results are in accordance with the findings of Zhao et al.,[12] where, under several pH and temperature conditions of induced autolysis, uridine is the most abundant ribonucleosides, followed by adenosine. In our study, the autolysis took place in real enological conditions, with low pH at 2.9−3.0, relative low temperatures (16 °C), and high CO_2 pressure (>6 atm) for 4 years. Induced autolysis in the various previous studies probably did not reproduce the autolytic lees process that occurs in the bottle under real conditions. Thus, in the current study (Figure 3 and Table 1), it seems that the progressive formation of uridine, in contrast to the other nucleosides, was promoted. Yeasts are rich in enzymes that could hydrolyze nucleic acids, all of which could be involved in the autolytic degradation of RNA in nucleotides, nucleosides, and nucleobases.[12] Nucleotidases act on nucleotides to release phosphate and nucleoside, while nucleosidases act on nucleosides to release nucleobases. In real enological conditions nucleotidases may be active at these temperatures and pH, while nucleosidases appear not to be very active. The three nucleosides determined (uridine, adenosine, and guanosine) may result from the degradation of nucleotides that occurs inside cells due to the enzymatic activity. However, to verify this point, a detailed study to find how nucleic acids are degraded (poly- oligo- or mononucleotides) over time inside the lees is needed.

During aging, uridine increased its content, and a relationship was established between uridine content and aging time ($R^2 > 0.8$) (Figure 3). The maximum concentration was found in the last months of aging reaching 28.4 mg L^{-1} and 18.1 mg L^{-1} at month 48 for series A and B, respectively, and C 22.2 mg L^{-1} at month 39 for series C (Table 1). No uridine kinetic differences between the three series were found. The uridine increment, according to the quality categories of Cava (Cava, Cava Reserva, and Gran Reserva), accounted for nearly 12% in each category. Uridine is used as a parameter to monitor freshness in other foodstuffs.[24] Our results suggest that uridine in sparkling wine could be used as a marker to monitor long-term aging.

Table 2. Evolution of Catabolites Content (mg/L) of Cava Wine During Aging without Lees

series	17 months	21 months	21 months	30 months	48 months
			Adenosine		
Ac	<0.01a[a]	<0.01a	<0.01a	<0.01a	<0.01a
Bc	<0.02a[b]	<0.02a	<0.02a	<0.02a	<0.02a
			Guanosine		
Ac	<0.06a[a]	<0.06a	<0.06a	<0.06a	<0.06a
Bc	<0.06a	<0.06a	<0.06a	<0.06a	<0.06a
			Uridine		
Ac	22.06a ± 0.60	22.17a ± 0.60	22.66a ± 0.61	23.20a ± 0.63	23.28a ± 0.63
Bc	14.12a ± 0.38	14.07a ± 0.38	14.95b ± 0.40	15.20b ± 0.41	14.95b ± 0.40
			Hipoxanthine		
Ac	<0.44[b]	<0.44	<0.44	<0.44	<0.44
Bc	1.01a ± 0.09	1.03a ± 0.10	1.12a ± 0.10	1.03a ± 0.10	1.17a ± 0.10
			Xanthine		
Ac	10.30a ± 0.33	10.00a ± 0.32	10.02a ± 0.32	9.74a ± 0.31	9.80a ± 0.31
Bc	10.54ab ± 0.34	10.16a ± 0.33	11.65c ± 0.37	11.33c ± 0.36	11.05bc ± 0.35
			Uric Acid		
Ac	0.62a ± 0.06	0.82b ± 0.08	0.71ab ± 0.07	0.70ab ± 0.07	0.77ab ± 0.08
Bc	0.74a ± 0.07	0.68a ± 0.07	0.81a ± 0.08	0.76a ± 0.08	0.83a ± 0.08

[a]<LOD. [b]<LOQ. Different letters indicate statistically differences by ANOVA.

Adenosine and guanosine behaved differently (Table 1). These nucleosides were not detected in base wine and did not appear while fermentation takes place (3 months). Adenosine concentration behaved in a similar way in the three series: it was found six months after bottling, once aging started. Its highest concentration was detected between 9 and 21 months (Cava and Reserva) while in long-aging Cava (Gran Reserva) this nucleoside seemed to continue its degradation (Figure 2). Guanosine showed a similar trend (Table 1).

The release of nucleotides and nucleosides during yeast autolysis could act as flavoring agents.[13] Exploration of the nucleoside profiles and their impact in Cava quality should be studied from the standpoint of flavor enhancers.

Quantification of Purines in Cava Wine During Aging "sur Lie". Purines have been studied in other foodstuffs as a flavor enhancer,[25] and they may also contribute to flavor in the evolution of Cava during aging and storage. Xanthine, hypoxanthine, and uric acid were detected in all base wines (Table 1). Xanthine is the main purine quantified in wine and sparkling wine. These purines increase their content until the end of second fermentation. The average of the percent increases (calculated at 3 months with respect to 0 months of the 3 series) corresponds at 11% of xanthine, 36% of uric acid, and more than double of hypoxanthine, and their concentration remains constant in sparkling wines even within the quality categories (Cava, Reserva, and Gran Reserva). These purines appear already in base wines, probably from the first fermentation, and do not appear to alter significantly during *sur lie* aging. Xanthine is a product on the pathway of purine degradation (Figure 2). It is created from guanine by guanine deaminase (EC 3.5.4.3.);[26] a previous step to guanine was needed by a purine-nucleoside phosphorylase (EC 2.4.2.1).[22] Xanthine comes from hypoxanthine too (hypoxanthine is a spontaneous deamination product of adenine)[27] by a xanthine dehydrogenase (EC 1.17.1.4.)[23] or by a xanthine oxidase (EC 1.17.3.2.).[23] Another possible pathway comes from xanthosine by the purine-nucleoside phosphorylase (EC 2.4.2.1.).[22] However, as seen in Figure 2, xanthine could be transformed to uric acid by the enzyme action of the xanthine dehydrogenase (EC 1.17.1.4.)[23] or by the xanthine oxidase (EC 1.17.3.2.)[23] mentioned above. However, Pantazopoulou and Diallinas[28] indicate that *Saccharomyces cerevisiae* lacks all the enzymes necessary for purine oxidation.

Another possible origin of sparkling wine purines could be from ATP degradation as described in the muscle tissue of seafood.[29] The post mortem sequence could be represented by the following cascade of decomposition: ATP → ADP → AMP → IMP → inosine → hypoxanthine → xanthine.

Quantification of Catabolites During Aging without Lees. To establish the role of lees in these wine catabolites, the lees of cava series (A and B) were removed at 9 months by means of the disgorging, and the corresponding sparkling wines (Ac and Bc series) were obtained and stored in the same cellar conditions. This procedure was performed in order to compare the catabolite contents between the wines in contact with lees and those without lees. The catabolites could be produced inside the cell and then be released from the lees to wine through the cell wall. The alternative would be the release of precursors (nucleotides) and enzymes to wine, and then the enzyme reactions produced these nucleosides and purines in the wine medium.

Table 2 shows the concentration of catabolites determined in sparkling wine aged without lees from month 13 to month 48. Adenosine and guanosine were not detected in either Ac series or Bc series.

Uridine, xanthine, hypoxanthine, and uric acid did not significantly increase their content during aging without lees. These results indicate that the presence of these nucleosides and purines was related to lees autolysis and not to chemical or enzymatic changes in the wine. The nucleosides detected in the present study derived mostly from RNA, which may remain the only source in the case of uridine, with rRNA being the most abundant of the different kinds of RNA.[30] Along these lines, Tudela et al.[16] described that cytoplasm content was already degraded at 9 months and ribosomes were present in the periplasmic space of lees. The determined breakdown products could be released into the extracellular environment (sparkling wine) by the degradation of the cell wall and the breakages of plasma membrane that occur during yeast autolysis. Figure 2 shows three TEM images for three cell states: cell fermentation

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(1 month) and lees at 12 months and at 48 months. The microfibrous material of the cell wall (CW) appeared more diffuse, the amorphous midzone of the inner wall layer is degraded, and from the ninth month onward the cells were plasmolyzed. During *sur lie* aging, the lees at different stages suffer cytoplasmic degradation accompanied by extensive loss and disorganization of the intracellular organelles (C).

In conclusion, only some RNA catabolites increase in sparkling wine during *sur lie* aging. When lees are removed these catabolite levels remain unchanged in wine medium. Thus, nucleoside content could be associated with cell degradation, which involves intracellular hydrolytic enzymes that act to release cytoplasmic and cellular compounds into the wine. In this sense, the aging in contact with lees has been revealed to be highly significant for sparkling wine: uridine amounts increase progressively during second fermentation and aging, and it could be used as autolysis marker. Purines appear as fermentative catabolites, which derived from nucleosides or ATP degradation. Further studies about the role played by nucleotides, or RNA, or DNA fragments in autolysis are needed.

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Notes

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