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Influence of Fatty Acids on Wine Foaming

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The influence of fatty acids (free and bound as ethyl esters) on wine foaming was studied in different white wines and the corresponding sparkling wines. Moreover, from three of these wines the foam formed by CO_2 injection was separated, and two fractions were then obtained: foam wine (FW) and remainder wine (RW). In these fractions and the sparkling wines produced from them, foam properties and fatty acids were also determined. The free fatty acids C8, C10, and C12 were negatively correlated with foamability (HM), whereas the ethyl esters of hexanoic, octanoic, and decanoic acids were positively related to HM. The value of HM was directly proportional to the ratio of esterified to unesterified fatty acids. This was confirmed by the changes that occur in the esterification ratio during the second fermentation and aging. No influence was observed on either the Bikerman coefficient or the stability time of foam.

KEYWORDS: Foam; fatty acids; ethyl esters; esterification ratio; wine; sparkling wine; fractionation; foam wine

INTRODUCTION

Several papers report the influence of factors such as grape variety, harvest, *Botrytis* infection, process, and aging on the chemical composition of wine and foam (*1*−*26*).

Some authors have established relationships between foaming properties and chemical compounds (*4*, *12*, *13*, *15*, *17*, *19*, *22*−*33*). Other authors, however, separated the foam and then studied its composition. They found that some compounds, such as amino acids (proline, alanine, and lysine), polysaccharides, metals such as iron, lipids, and proteins, are enriched in foam (*2*−*4*, *6*, *7*, *14*). In other studies, the chemical compounds were added to the sample in different concentrations to study how the foaming parameters were modified (*1*, *4*, *11*).

The influence of lipids on foam has mostly been studied in beer and, depending on their concentration, the interactions with other compounds such as proteins (*34*−*37*). Bosch et al. (*2*) report, in must, that lipids accumulate in the foam, reducing surface tension and stabilizing it. In wines, research done on the possible relationships among lipid content, fatty acids, and foam behavior has produced contradictory findings. The addition of octanoic and decanoic fatty acids has been found to have a negative effect on the foam stability time, but it positively influences foam collar height (*4*). Dussaud et al. (*11*) added a lipid mixture to wine and found that the foam was not affected. However, when they reduced the ethanol concentration, they found this had an adverse effect on bubble lifetime. Pueyo et al. (*13*) argued that the total content of linolenic acid and total content of palmitic acid were, respectively, the best indicators of foam stability in wine and of foam height in Cava, both having a positive influence. These conflicting results regarding the influence of fatty acids on foam may be a result of the quantification method used to measure fatty acids (*38*) and/or their interaction with other compounds, which may also be affected by different factors.

To evaluate the importance of fatty acids (free and bound as ethyl esters) for the foam properties of wines, we applied the following procedures. First, in base wine, we determined the free fatty acid and ethyl ester contents as well as the foam parameters. Correlation analysis was used to establish possible relationships between the compounds analyzed and the foam measurements. Then, the foam formed during CO_2 injection was separated. Two fractions of each total or initial wine (TW) were then obtained: foam wine (FW) and remainder wine (RW). The fatty acids, ethyl esters, and foam parameters were subsequently determined in TW, FW, and RW, and the enrichment equations of Brissonnet and Maujean (*6*) were applied. Finally, to corroborate the previous results, we also examined sparkling wine. This meant that the evolution of fatty acid and ethyl ester concentration and foam was considered during the second fermentation and aging. All of the base wines and the corresponding sparkling wines were produced on an industrial scale.

MATERIALS AND METHODS

Samples. Fourteen base white wines from the Cava region were used for correlation analysis. From six of these base wines, sparkling wines (Cavas) were obtained.

Cava is a quality sparkling wine produced in a specific region (v.e.c.p.r.d.), using the traditional “méthode champenoise”. The second alcoholic fermentation takes place inside the bottles, after the addition

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Table 1. Correlation Coefficients (*r*) between Foam Parameters [Foamability (HM), Bikerman Coefficient (Σ), and Stability Time of Foam (TS)] and Free Fatty Acids and Ethyl Esters

		HM	Σ	TS
	median (min–max)[a]	92 (34–220)	17 (14–28)	205 (20–510)
C6 ($n = 28$)	5257 (4424–8008)	ns[b]	ns	ns
C8 ($n = 28$)	10325 (8074–17551)	–0.4344	ns	ns
C10 ($n = 28$)	2377 (953–3619)	–0.6561	ns	ns
C12 ($n = 28$)	86 (61–122)	–0.5691	ns	ns
C14 ($n = 28$)	24 (15–48)	ns	ns	ns
C16 ($n = 28$)	190 (84–288)	ns	ns	ns
C18	<QL[c]			
ethyl hexanoate ($n = 28$)	1686 (711–2628)	0.6466	ns	ns
ethyl octanoate ($n = 28$)	1984 (814–3952)	0.8624	ns	ns
ethyl decanoate ($n = 28$)	388 (138–673)	0.8962	ns	ns
ethyl dodecanoate ($n = 15$)	21 (14–54)	ns	ns	ns
ethyl myristate	<QL			
ethyl palmitate	<D[d]			
ethyl stearate	<DL			

[a] Median and minimum–maximum values of the concentrations of fatty acids and ethyl esters (μg/L), HM (mm), Σ (s), and TS (s). [b] Nonsignificant result ($p > 0.05$). [c] QL, quantification limit. [d] DL, detection limit.

Table 2. Mean Values of Foam Parameters ($n = 3$) and Fatty Acids Concentrations ($n = 3$) of Wines Obtained by Fractionation

	TW1[a]	FW1[b]	RW1[c]	%E1[d]	TW2[a]	FW2[b]	RW2[c]	%E2	TW3[a]	FW3[b]	RW3[c]	%E3
foam parameters[e]												
HM	72	105	33		52	83	47		62	87	45	
Σ	12	14	19		30	14	28		37	16	27	
TS	350	78	465		384	5	224		290	6	198	
free fatty acids												
C6	5228	6223	4693	14	3804	4723	3481	15	4485	4725	3516	15
C8	8904	10829	7495	18	7789	8461	6619	12	8247	9091	6214	19
C10	723	1016	307	54	1543	2175	524	61	1502	2258	556	60
C12	25	21	23		152	169	149		205	221	210	
C14	30	27	25		30	35	27		29	30	28	
C16	152	171	98	27	560	934	480	32	245	300	157	31
C18	<LQ[f]	<LQ	<LQ		<LQ	<LQ	<LQ		<LQ	<LQ	<LQ	
ethyl esters												
hexanoate	1139	1057	999		877	101	436		1178	167	675	
octanoate	1325	1350	1068		634	462	321		836	476	441	
decanoate	81	131	16	78	151	286	17	89	163	228	29	78
dodecanoate	<LQ	<LQ	<LQ		<LQ	<LQ	<LQ		<LQ	<LQ	<LQ	
myristate	<LQ	<LQ	<LQ		<LQ	<LQ	<LQ		<LQ	<LQ	<LQ	
palmitate	<LQ	<LQ	<LQ		<LQ	<LQ	<LQ		<LQ	<LQ	<LQ	
stearate	<LQ	<LQ	<LQ		<LQ	<LQ	<LQ		<LQ	<LQ	<LQ	

[a] TW, concentration in initial of total wine (μg/L). [b] FW, concentration in foam wine (μg/L). [c] RW, concentration in residual wine (μg/L). [d] %E, enrichment percentage of fatty acids (Cn) and ethyl esters (EEn) in foam wine. [e] HM, foamability (MM); Σ, Bikerman coefficient (s); TS, stability time of foam (s). [f] LQ, quantification limit.

to the base wines of sugar and selected yeasts. Only after 9 months of aging in contact with yeasts are sparkling wines considered to be Cava by the Spanish Certified Appellation of Origin [Denominación de Origen controlada (DOC)].

Three other base wines (TW1, TW2, and TW3) were used for fractionating as described below. The three wine samples obtained by the fractionation of the first wine (TW1, FW1, and RW1) were also fermented again to obtain the corresponding sparkling wines (applying the process described before).

Aging at zero (before addition of “tirage liqueur”), 3, 6, and 9 months was considered in duplicate. Two bottles from each sampling point were analyzed separately to take into account the variation between them. Samples were stored at 18 °C until analysis.

The analytical characteristics of base wines were within the following intervals: alcohol content, 10−11.5% (v/v); titratable acidity, 5.5−7.0 g/L of tartaric acid; volatile acidity, 0.20−0.25 g/L of acetic acid; and total SO_2, 70−90 mg/L.

Fractionating. The foam was made by injection of CO_2 with a constant flow (7 L/h) and under a constant pressure (100 kPa), using Mosalux equipment (*4*). The foam was then separated using a suction pump. Two fractions were thus obtained from each initial or total wine (TW1, TW2, and TW3): the foam wine (FW1, FW2, and FW3) and the wine remaining after foam separation (RW1, RW2, and RW3). Enological parameters of these resulting wines were comparable to those of the base wines.

Table 3. Correlation Coefficients (*r*) between Foam Parameters [Foamability (HM), Bikerman Coefficient (Σ), and Stability Time of Foam (TS)] and the Esterification Ratios of the Fatty Acids (ERCn)[a]

esterification ratio of	HM	Σ	TS
C6 ($n = 28$)	0.6230	ns	ns
C8 ($n = 28$)	0.7917	ns	ns
C10 ($n = 28$)	0.7507	ns	ns
C12 ($n = 11$)	ns[b]	ns	ns
sum (C6 + C8 + C10) ($n = 28$)	0.8229	ns	ns

[a] ERCn:(EEn/Cn) × 100: (ethyl ester concentration/fatty acid concentration) × 100. [b] Nonsignificant result ($p > 0.05$).

Analytical Methods. Foam measurements were performed using Mosalux equipment and the Maujean et al. (*4*) procedure, following the method of Gallart et al. (*39*). Samples were analyzed immediately after collection. Foamability (HM), the Bikerman coefficient (Σ) (*8*), and foam stability time (TS) were measured: HM represents the wine’s ability to foam; Σ represents the average bubble lifetime, at a constant foam collar height, when foam formation and destruction are balanced; and TS is the time until all bubbles collapse, after gas injection is stopped.

Fatty acids and ethyl esters were determined by GC, according to the method described by Gallart et al. (*38*).

Table 4. Evolution of Foamability and Esterification Ratio during the Second Fermentation and Aging

	Cava TW1[c]	Cava FW1[d]	Cava RW1[e]	Cava 1	Cava 2	Cava 3	Cava 4	Cava 5	Cava 6
0 months (base wine)									
HM[a]	73	105	34	128	191	191	218	179	103
ER (C6 + C8 + C10)[b]	17.6	18.0	16.2	44.5	32.4	34.2	40.4	32.3	30.1
3 months									
HM	43	73	30	62	70	90	107	81	79
ER (C6 + C8 + C10)	12.9	10.8	11.2	28.8	21.5	22.6	27.6	28.1	24.9
9 months (Cava)									
HM	23	41	18	62	66	50	86	53	64
ER (C6 + C8 + C10)	8.1	7.7	5.7	25.8	24.8	22.0	18.3	17.1	19.3

[a] Mean values of foamability (mm) ($n = 3$). [b] Mean values of the esterification ratio (%) of the sum of C6, C8, and C10 ($n = 3$). [c] TW, initial or total wine. [d] FW, foam wine. [e] RW, residual wine.

All experiments were performed in triplicate.

Enrichment Study. The Brissonnet and Maujean (*6*) equation was applied:

$$E\% = \frac{FW - [(FW + RW)/2]}{(FW + RW)/2} \times 100$$

E% is the enrichment percentage in foam wine, and FW and RW are the concentrations of each analyzed compound in the foam wine and in the remainder wine, respectively.

Statistical Analysis. Statgraphics 7.0 (*40*) was used for analyzing the statistical data. Correlation analysis was carried out between foam characteristics (HM, Σ, and TS) and fatty acids (free fatty acids, ethyl esters, and esterification ratios). In the enrichment study, an ANOVA was used to establish differences ($p < 0.05$) between TW, FW, and RW of the three wines fractionated.

RESULTS AND DISCUSSION

Foamability (HM) was negatively correlated with the presence of the free fatty acids C8, C10, and C12. In contrast, there was a positive correlation with HM (**Table 1**) when the fatty acids were esterified as ethyl esters. Therefore, the influence that fatty acids have on foamability seems to depend on their chemical form (free or esterified), and this could also explain the contradictory results of previous studies. No correlation was observed between the fatty acids and ethyl esters and the other foam parameters studied (Σ and TS) (**Table 1**).

Table 2 shows the results ($n = 3$) for foam parameters (HM, Σ, and TS) in the fractions. Mean values of the coefficients of variation were 4.8 and 7.0%, respectively. Foam wine (FW) presents significantly higher values of HM than initial wine (TW), the difference being even greater when FW is compared with residual wine (RW). In contrast, the parameters TS and Σ in residual wines (RW) were always higher than those in foam wine (FW). HM and TS values were in agreement with those obtained by Brissonnet and Maujean (*6*), in that the foam characteristics of the two fractions obtained (FW and RW) differ with respect to initial wine (TW).

Table 2 also shows the results for fatty acids enrichment in foam wine (E%). The Brissonnet and Maujean equation (*6*) was applied only when the concentration in foam wine (FW) was significantly higher, and the concentration in residual wine (RW) significantly lower, than the concentration in initial wine (TW), considering the analytical variation of each fatty acid. The differences between the concentrations of the C12 and C14 fatty acids in the fractions were nonsignificant, because they were similar or lower than their coefficients of variation (8.9 and 10.2%). The fatty acids C6, C8, C16, and particularly C10 and its ethyl ester were found to be enriched in foam wine. Moreover, the enrichment percentages were similar for the three wines studied. The ethyl esters of C6 and C8 were lost during fractionation, as their amounts in FW and RW were lower than those in TW.

The negative relationship found in the correlation analysis (**Table 1**) between free fatty acids and foamability may seem to contradict the fact that they were enriched in foam wine (**Table 2**). It is possible that the influence of fatty acids depends on the different interactions established by the carboxylic group (free fatty acid) or by the carbonylic group (ethyl ester) with other wine compounds. To test this hypothesis, the esterification ratio of each fatty acid (ERCn) was calculated for each of the 14 base wines, as a quotient between the ethyl ester concentration (EEn) and the fatty acid concentration (Cn): $[(EEn/Cn) \times 100)]$. The correlations between the ERCn and HM are shown in **Table 3**: it can be seen that they were always positive and that the correlation coefficient (r) was >0.60. Although the fatty acids and ethyl esters migrated to foam, the HM value increased when the proportion of esterified fatty acids was higher.

The fatty acid esterification ratio changed for the second fermentation and aging during 9 months. **Table 4** shows that in all of the wines studied, both the foamability and the esterification ratio of the sum of C6, C8, and C10 decreased significantly during the second fermentation and 9 months of aging (mean values of the coefficients of variation were 6.6% for foamability and 2.5% for the esterification ratio of the sum of C6, C8, and C10). The decrease of the esterification ratio of the sum of C6, C8, and C10 could be due to a significant decrease of the levels of the ethyl esters of the different fatty acids, mainly the ethyl octanoate. The concentrations of the free fatty acids did not change or decreased slightly during the second alcoholic fermentation and 9 months of aging (data not shown). Also, from a comparison of TW1, FW1, and RW1, it can be seen that HM was always significantly greater during aging in foam wine (FW), although the differences between fractions are less than those prior to starting the second fermentation (0 months) (**Table 4**).

In conclusion, we believe that the influence fatty acids have on foamability in wine and sparkling wine depends on the equilibrium between the free and bound (as ethyl ester) forms. Thus, all wine-making procedures, which increase the synthesis of ethyl esters from fatty acids, will benefit the wine's foamability.

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