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Quantitative survey of microflora in shelf wines

by

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Eine quantitative Erfassung der Mikroflora von Flaschenweinen aus dem Einzelhandel

Zusammenfassung. — An 310 in Einzelhandelsgeschäften zum Verkauf anstehenden Weinflaschen, die aus 21 Weinbauländern stammten, wurde eine quantitative mikrobiologische Bestandsaufnahme durchgeführt. Die geprüften Weine umfaßten folgende Typen: Leichte Weine, Perlweine, Roséweine, Traubenschaumweine, rote Tafelweine, weiße Tafelweine, Sekte, Apéritifs, Wermutweine, Würzweine, Apfelweine, weinhaltige Mischgetränke und Obstweine. Jeder Weintyp wird durch folgende Daten charakterisiert: 1. Minimale und maximale Anzahl der lebensfähigen Keime von Schimmelpilzen, Bakterien und Hefen; 2. jeweilige Häufigkeitsverteilung der Keimzahlen von Schimmelpilzen, Bakterien und Hefen; 3. Häufigkeitsverteilung verschiedener Kombinationen von Mikroorganismentypen, die aus derselben Flasche isoliert wurden; 4. Relation von Hefen- und Bakterienzahlen in Weinen, die beide Mikroorganismentypen enthielten.

Introduction

Our laboratory is investigating the biological stability of commercial bottled wines. Review literature (1, 5, 7) indicates a lack of emphasis on quantitative data concerning microflora in retail shelf wines. In the last decade, more attention has been given to procedures for determining viable counts in wines (2, 6, 8, 9). Surprisingly, in spite of the interest in viable count procedures, there is a scarcity of actual published data on the final shelf product. Data on shelf products is currently very relevant since some countries are severely scrutinizing bottled wines for various components, additives and viable counts (9). The present preliminary survey enumerates the numbers of viable moulds, yeasts and bacteria in some domestic and foreign wines selected from the retailer.

Experimental

The wine categories, countries of origin and number of bottles tested are presented in Table 1. In the crackling, rosé, sparkling and low alcohol categories, three bottles (from the same case) of each brand were tested. In all other categories, one bottle of each brand was tested.

Viable counts were determined by membrane (0.45 microns) filtration (3). Membranes were incubated at 27 °C up to 3 days on the following Difco (4) media: Micro Assay Culture Agar; Malt Extract Agar; Fluid SABOURAUD (Glucose) Medium plus 2% (w/v) agar; Wort Agar; W. L. Medium; and YM Agar. Since no medium consistently supported growth, the viable counts quoted are those on the medium yielding the highest count for the type of micro-organism (mould, yeast or bacterium) in question.

Results

The ranges of viable mould, bacterium and yeast counts are presented in Table 2. Frequencies of viable mould, bacterium and yeast counts are presented in Tables 3, 4 and 5 respectively. Frequencies of different combinations of types of viable micro-organisms present in the same bottle are presented in Table 6. The relation

between yeast and bacterium counts in wines containing both types of micro-organisms is presented in Table 7.

Discussion

For various commercial reasons, the complete details concerning the production history and stabilization procedure(s) for each product cannot be included in this report. However, since this preliminary survey, our laboratory and certain industries have co-operated in accounting for and reducing the viable counts in various products. Based on this co-operative experience, we offer several comments and observations.

The microflora in any particular shelf bottle can differ quantitatively and qualitatively from microflora in bottles derived from the same case and from the same bottling run. The presence of high numbers of moulds, yeasts, or bacteria in one bottle didn't necessarily correspond to a similar high count in another bottle from the same case and bottling run. Also, the same combination of micro-organisms (see Table 6) in any one bottle did not necessarily occur in another bottle from the

Table 1

Wine type, origin and number of bottles tested
Weintyp, Herkunft und Anzahl der untersuchten Flaschen

Country of origin	Number of bottles tested												
	Low Alcohol	Crackling	Rosé	Sparkling	Red Table	White Table	Champagne	Apéritif	Vermouth	Flavoured Wine	Cider	Wine Cocktail	Fruit Wine
Argentina					1								
Australia			3			1							
Bulgaria					1	1							
Canada	33	42	36	27	27	21	8	2	9	2	2	2	6
Chile						1	1						
Denmark												1	
France			3	3	7	5	1	1	1				
Germany				3	1	2							
Greece					1	1							
Hungary					1	1							
Israel			3	3	1	1							
Italy			3		1	2		1	2				
Jugoslavia					1	1							
Portugal		3	3		1	1		1					
Rumania						1							
South Africa		3		3	1	1							
Spain				3	1	1				2			
Switzerland					1	1							
Turkey					1	1							
United Kingdom								1			2	1	1
United States				3	1	1							

Table 2

Frequency of viable mould counts in bottled wines
Anzahl lebensfähiger Keime von Schimmelpilzen, Bakterien und Hefen in Flaschenweinen

Wine type	Range of counts per 100 ml		
	Moulds	Bacteria	Yeasts
Low alcohol wine	0 — 180	0 — 112	0 — 2
Crackling	0 — 60	0 — 100	0
Rosé	0 — 22	0 — (400)	0
Sparkling	0 — 150	0 — 104	0 — 232
Red table wine	0 — 251	0 —(1000)	0 —(7000)
White table wine	0 —(300)	0 —(1200)	0 — 518
Champagne	0 — 6	0 — (400)	0 — 52
Apéritif	0 — 68	0 — 24	0 — 12
Vermouth	0	0 — 42	0
Flavoured wine	0 — 2	0 — 238	0 — 8
Cider	0	0 — 20	0 — 14
Wine cocktail	0	2 — 636	0 — 2
Fruit wine	0 — 10	0 — 384	0 — 24

Bracketed numbers indicate estimated counts.

Table 3

Frequency of viable mould counts in bottled wines
Häufigkeitsverteilung der Keimzahlen von Schimmelpilzen in Flaschenweinen

Range of counts per 100 ml	Number of bottles containing various counts												
	Low Alcohol	Crackling	Rosé	Sparkling	Red Table	White Table	Champagne	Apéritif	Vermouth	Flavoured Wine	Cider	Wine Cocktail	Fruit Wine
0	22	37	35	29	21	19	4	3	12	3	4	4	5
1— 10	8	10	14	12	23	16	6	2		1			2
11— 20			1	2	1	4							
21— 30	1		1										
31— 40													
41— 50						1							
51— 60		1											
61— 70								1					
71— 80													
81— 90	1			1									
91—100													
101—200	1			1	2	3							
201—300					1								
301—400						1							

same case and from the same bottling run. Essentially, this means that the viable count in a single shelf sample may be representative of only that sample itself and have no bearing on the population of bottles comprising one bottling run. None of the above results is surprising in view of the fact that in our sanitation surveys, we have encountered variations in numbers and types of micro-organisms in different bottling spouts, empty bottles as well as unused caps. We therefore suggest that industry adopt representative sampling techniques for their quality control programs. Without a proper sampling technique, it is difficult to organize an effective routine for monitoring viable counts in bottled products. Also, without representative sampling, the laborious task of classifying micro-organisms in a single shelf bottle can be an exercise in irrelevancy as far as explaining the presence of the micro-organisms is concerned. For instance, we noted that some viable counts were due to the bottling process (i.e. spouts, empty bottles and unused caps) and not due to the pre-bottling history of the wine.

Regarding stabilization procedures, there was no exclusive relationship between viable count and type of biological stabilization procedure. Commercial

Table 4

Frequency of viable bacterium counts in bottled wines
Häufigkeitsverteilung der Keimzahlen von Bakterien in Flaschenweinen

Range of counts per 100 ml	Number of bottles containing various counts												
	Low Alcohol	Crackling	Rosé	Sparkling	Red Table	White Table	Champagne	Apéritif	Vermouth	Flavoured Wine	Cider	Wine Cocktail	Fruit Wine
0	14	18	24	27	9	13	2	2	4	1	2		4
1— 10	10	13	15	12	21	18	2	2	3	1	1	2	1
11— 20	3	7	2	3	8	3	1			1	1		
21— 30		3	1		2	2		2	2				
31— 40	1	5	1	1	1	3	1		1				1
41— 50	2	1							2				
51— 60			2	1									
61— 70	1				1	1							
71— 80	1		2				1						
81— 90						1							
91— 100		1	2		1								
101— 200	1		1	1	1		1						
201— 300					1		1			1		1	
301— 400			1		1		1						1
401— 500					1								
501— 600						2						1	
601— 700													
701— 800													
801— 900													
901—1000													
1001—2000						1							

wines are seldom biologically stabilized by a single chemical or physical procedure. Hence, any attempt to relate the presence of yeasts, bacteria and moulds in a bottled product must scrutinize the individual and collective effect(s) of all measures applied to the wine and all equipment that comes in contact with the wine. For instance, we have found that a high count in a membrane filtered wine may not be due to a fault in the membrane filter system *per se* but to post-membrane filter contamination. This same situation can apply to pasteurized wines where the product acquires microflora after the pasteurizer.

While viable counts can be difficult to explain, the absence of viable micro-organisms can also be difficult to explain. For example, the absence of micro-organisms in a membrane filtered or pasteurized product may not necessarily be due to the membrane filter or the pasteurizer but to the preservative(s) in the product.

Table 5

Frequency of viable yeast counts in bottled wines
Häufigkeitsverteilung der Keimzahlen von Hefen in Flaschenweinen

Range of counts per 100 ml	Number of bottles containing various counts												
	Low Alcohol	Crackling	Rosé	Sparkling	Red Table	White Table	Champagne	Apéritif	Vermouth	Flavoured Wine	Cider	Wine Cocktail	Fruit Wine
0	32	48	51	42	37	28	2	2	12	3	2	2	3
1— 10	1			1	5	11	5	3		1	1	2	3
11— 20						3		1			1		
21— 30					2		1						1
31— 40					1								
41— 50							1						
51— 60				1			1						
61— 70					1								
71— 80													
81— 90													
91— 100						1							
101— 200													
201— 300				1									
301— 400													
401— 500													
501— 600						1							
601— 700													
701— 800													
801— 900													
901—1000													
1001—2000					1								
2001—3000													
3001—4000													
4001—5000													
5001—6000													
6001—7000					1								

This survey also indicated that there is no mutually exclusive relationship *per se* between the viable mould count and type of bottle closure. Bottles with bark cork closures may or may not have high mould counts. Bottles with plastic closures may or may not have high (even TNTC) mould counts.

This survey indicates the numbers and types of microflora in some domestic and foreign retail shelf wines. Recent trends are not encouraging chemical stabilization for foods and beverages. In view of the increasing scrutiny of wines for viable counts, this report should indicate to some of the world's wine industries, the effectiveness of their current biological stabilization procedures.

Summary

A quantitative microbiological survey was conducted on 310 shelf bottles from 21 wine producing countries. The wines examined included the following types: low alcohol; crackling; rosé; sparkling; red table wine; white table wine; champagne; apéritif; vermouth; flavoured wine; cider; wine cocktail; and fruit wine. Data on each wine type is presented as (I) range of viable mould, bacterium and yeast counts, (II) frequency of individual mould, bacterium and yeast counts, (III) frequency of different combinations of types of micro-organisms isolated from the same bottle and (IV) relation between yeast and bacteria counts in wines containing both types of micro-organisms.

Table 6

Combinations of types of micro-organisms (moulds, bacteria, yeasts) in bottled wines
Kombinationen von Mikroorganismentypen (Schimmelpilze, Bakterien, Hefen) in Flaschenweinen

Combination	Number of bottles containing various combinations												
	Low Alcohol	Crackling	Rosé	Sparkling	Red Table	White Table	Champagne	Apéritif	Vermouth	Flavoured Wine	Cider	Wine Cocktail	Fruit Wine
No viable count	9	16	17	17	3	10	1	0	4	1	1	0	0
Moulds + bacteria + yeasts	1	0	0	1	2	13	6	1	0	1	0	0	0
Moulds + bacteria only	5	9	9	6	19	9	0	1	0	0	0	0	0
Moulds + yeasts only	0	0	0	1	0	1	0	0	0	0	0	0	0
Bacteria + yeasts only	0	0	0	0	8	2	1	2	0	0	1	2	2
Moulds only	5	2	7	8	6	2	0	1	0	0	0	0	2
Bacteria only	13	21	18	11	9	7	1	0	8	2	1	2	1
Yeasts only	0	0	0	1	1	0	1	1	0	0	1	0	2

Table 7

Relation between yeast and bacteria counts in bottles containing both types of micro-organisms

Relation zwischen Hefen- und Bakterienzahlen in Flaschen, die beide Mikroorganismentypen enthalten

Wine type	Bottle No.	Viable counts per 100 ml		
		Moulds	Bacteria	Yeasts
Low alcohol	1	2	36	2
Sparkling	2	16	104	10
Red Table Wine	3	—	1	1
	4	—	25	24
	5	—	19	4
	6	—	347	3
	7	—	11	67
	8	2	2	2
	9	—	291	37
	10	—	(1000)	(7000)
	11	—	96	1120
	12	155	8	27
White Table Wine	13	—	2	2
	14	—	504	518
	15	2	12	2
	16	2	2	4
	17	150	2	2
	18	20	2	2
	19	4	2	2
	20	172	4	20
	21	16	(600)	8
	22	2	20	16
	23	6	4	2
	24	4	4	2
	25	42	(1200)	16
	26	2	26	100
	27	6	4	2
Champagne	28	6	128	52
	29	—	6	6
	30	4	244	48
	31	2	16	8
Apéritif	32	—	2	2
	33	68	4	12
	34	—	24	2
Flavoured Wine	35	2	18	8
Cider	36	—	20	2
Wine Cocktail	37	—	2	2
Fruit Wine	38	—	296	2
	39	—	34	2
	40	—	384	2

Brackets indicate approximate counts.

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