Acta Alimentaria, Vol. 31 (4), pp. 343–354 (2002)

STUDY OF TOKAJI ASZÚ WINE FLAVOUR BY SOLID PHASE MICROEXTRACTION METHOD

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(Received: 12 December 2001; accepted: 9 June 2002)

In this study the role of different yeast strains in the production of volatile flavour components of Tokaji Aszú wine was tested. The effect of a *Saccharomyces cerevisiae* starter and that of the typical endogenous *Candida stellata* strain as well as spontaneous fermentation were studied and compared. For the fast comparison of aroma profile, a solid phase microextraction (SPME) sampling and a GC-MS separation and identification were used. Thirty of the present compounds were selected to characterise the changes of flavour. Significant differences were found between wines fermented with different yeast strains. Application of a *Saccharomyces cerevisiae* starter alone accelerated the fermentation but this caused only little change in the aroma profile and content. *Candida stellata* contributed weakly to the production of aroma, especially to that of the longer carbon chain ethyl esters. Characteristic compounds of aged wine were detected in bottle aged Tokaji Aszú. The change of aroma profile as a function of bottle storage time was studied. The concentrations of vitispirane, trimethyl dihydronaphtalene, 2-phenylethanol and diethyl succinate increased in the course of ageing time, while those of 3-methyl-butyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate and ethyl dodecanoate decreased.

Keywords: gas chromatography – mass spectrometry, solid phase microextraction (SPME), aroma compounds, wine, Tokaji Aszú, yeast

The famous Tokaji Aszú, the King of Wines, Wine of Kings is a late harvest wine made from Aszú berries shrivelled up by *Botrytis cinerea*. The raisin-like grapes are handpicked in a wooden butt, called "puttony", of a capacity of 20–25 kg. Three, 4 or 5 (occasionally 6) buts of Aszú-paste is added to one Gönci cask (136 l) of good quality, new dry wine, mixed and soaked for 1–2 days in order to extract the natural sugar content and flavours. The wine is then drawn off to ferment for a second time. The fermentation takes time because of the high sugar content and the low temperature of the cellars. The specifications require that Aszú wine must be aged for at least 2 years in cask and one in bottle before release.

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0139-3006/2002/$ 5.00

In the literature there are relatively few papers dealing with the flavour of Tokaji Aszú (SCHREIER et al., 1976; MIKLÓSY & PÖLÖSNÉ, 1997; MIKLÓSY et al., 2000; MURÁNYI & KOVÁCS, 2000). SCHREIER and co-workers (1976) used a pentane-dichloromethane liquid-liquid extraction for the isolation of flavour substances, while MIKLÓSY and PÖLÖSNÉ (1997) Freon extraction. The latter team identified numerous compounds, but only 40 of them were used for comparison. MURÁNYI and KOVÁCS (2000) used SPME for sampling the headspace.

SPME technique is widespread for the study of flavour substances (JIA et al., 1998). It is also used for fast screening of wine aroma (VAS, 1997; VAS et al., 1999, and WALLNER et al., 1999).

FRAILE and co-workers (2000) investigated the effect of *Saccharomyces cerevisiae* strains on the volatile composition of rosé wines. It was established that the formation and final concentration of the majority of volatile substances were determined by the strain dominating during fermentation. LEINO and co-workers (1993) used a dynamic headspace method for analysis of volatile compounds in heat-treated Chardonnay and Semillon wines.

The undesirable flavour compounds formed during bottle ageing were described among others by SCHREIER (1984), RAPP and VERSINI (1997).

The aim of this work was the study of fast screening of aroma profile of Tokaji wine using SPME to obtain information on the effect of different yeasts and bottle ageing. The papers of MIKLÓSY and PÖLÖSNÉ, (1997), MIKLÓSY and co-workers (2000) listed above delt only with young Tokaji Aszú.

1. Materials and methods

1.1. Fermentation with different yeasts

In the fermentation experiment, the starting wine was a five-butt, raw Aszú from 1999, prepared at the Royal Tokaj Wine Company, Mád. Spontaneous fermentation, the effect of a *Saccharomyces cerevisiae* starter and that of the typical endogenous *Candida stellata* strain were studied and compared at Szent István University. The original yeast biota of the base wine was removed by filtration and the wine was inoculated with the respective yeast strains, isolated earlier at Tokaj from fermenting wines. The wine with indigenous biota and the inoculated ones were fermented under microvinification conditions at 15 °C, in three replicates each. The samples studied are listed below:

A: spontaneous fermentation

B: after removal of the original biota inoculated with *Candida stellata* (ca. 106 cells/ml)

C: after removal of the original biota inoculated with *Candida stellata* (ca. 106 cells/ml) and *Saccharomyces cerevisiae* (ca. 104 cells/ml)

D: after removal of the original biota inoculated with *Saccharomyces cerevisiae* (ca. 104 cells/ml)

1.2. Spiking with diethyl disulphide

In order to study the ability of SPME sampling for the detection of volatile sulphur compounds, diethyl disulphide (SIGMA) was added to Tokaji wine in 1 mg l^{-1} and 10 μg l^{-1} concentration.

1.3. Effect of bottling time

Bottled Tokaji wine samples were obtained with courtesy of Crown Estates, Hungary and were produced at Megyer Rt., Sárospatak. Altogether 9 samples of different butt numbers from 1989 to 1999 were studied.

1.4. Solid phase microextraction

For the fast comparison of aroma profile, a SPME sampling was developed. A PDMS (polydimethylsiloxane) apolar fiber of 100 μm film thickness (Supelco, Inc.) was applied. A 15-ml wine sample was poured into a 40-ml screw cap vial with pre-drilled septa. Three vials were heated in a block thermostat at 40 °C, meanwhile they were slowly stirred with a magnetic stirrer. The vapour phase aroma profile was tested both at room temperature and at 40 °C, the latter was selected to speed up the equilibration and to standardise the conditions of headspace analysis (room temperature changed in a quite wide interval). The sample was equilibrated for 2 h before testing. The fiber was heated for 10 min before headspace adsorption. Adsorption and desorption time was 10 and 2 min, respectively. In microvinification samples, the SPME sampling was performed in two vials from three replicate treatments each. From bottled wines, sampling was accomplished at least in 3 replicate vials.

1.5. Gas chromatographic – mass spectrometric analysis

The separation and identification of volatile aroma components was carried out by gas chromatography-mass selective detection. A HP 5890 gas chromatograph coupled with a HP 5971 mass selective detector was used. The column was RH-5ms 30 m×0.25 mm i.d., 0.25 μm film thickness. Helium (purity 4.8) was used as a carrier gas. Splitless injection was set for 1 min. The oven temperature was set at 60 °C for 1 min and then programmed at 10 °C min^{-1} to 100 °C, in a second ramp at 15 °C min^{-1} to 200 °C for 8 min. Detector line temperature was 280 °C. The compounds were identified using Wiley 275 library. As no internal standard was added, the absolute peak areas were compared similarly to the paper of VAS (1997).

1.6. Sensory evaluation

Sensory evaluation of wine samples "A"-"D" was performed by 7 judges on a 25-point unweighed scale at St. István University, Department of Oenology.

1.7. Statistical analysis

Paired comparison of samples was performed using Student's t-test. Significant differences in fermentation experiments were determined at $P \leq 0.05$ level. In case of stored samples, regression analysis was accomplished.

2. Results and discussion

2.1. The effect of different yeasts

From the several hundred volatile components described in wine aroma (SCHREIER et al., 1976), about 50 were found, of which 30 were selected to characterise the changes of flavour profile. They were mainly esters due to the applied apolar SPME fiber coating but alcohols, acids, aldehydes were also detected as minor compounds. Among the sulphur compounds potentially present, only sulphur dioxide was found. The following monocarboxylic acid ethyl esters were identified: ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate, ethyl nonanoate, ethyl 9-decenoate, ethyl decanoate, ethyl dodecanoate, ethyl tetradecanoate, and ethyl hexadecanoate.

Hydroxy-, dicarboxylic and aromatic acid esters: ethyl lactate, diethyl succinate and ethyl phenylacetate. Acetate esters: hexyl acetate, octyl acetate and phenylethyl acetate.

Alcohols: 3-methyl-butanol, 1-hexanol, linalool, 2-phenylethanol. Aldehydes: acetaldehyde, benzaldehyde, nonanal and decanal. Acids: caproic, octanoic, decanoic acids. Miscellaneous: sulphur dioxide, BHT (as additive).

Sotolone, known to be synthesised by *Botrytis* was not found, possibly because of its high solubility in water and low concentration. Its characteristic fragment ions (83, 128, 57) were not detected in extracted ion chromatograms.

The chromatogram of sample "D", inoculated with *Saccharomyces cerevisiae*, is shown in Fig. 1. The peaks eluting after 3-methyl-butanol are numbered.

For the comparison of different treatments the absolute peak areas were used, similarly to VAS and co-workers (1999).

Significant differences were found between wines fermented by various yeast strains. Figs 2 and 3 show the difference in selected major and minor esters produced by different yeast biota. The high standard deviation results from replicate vinification treatments; the repeatability from the same sample is much better. Figure 4 presents the difference in alcohol-free total volatile compounds (the area of ethyl alcohol peak was not taken into account).

The wines inoculated with *Candida stellata* (B) and *Saccharomyces cerevisiae* (D) differed most. As linalool, nonanal, caproic acid and benzaldehyde were not detectable in "D", they were assumed to be formed by *Candida. Saccharomyces* produced more esters, namely ethyl butyrate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate (significant at $P \leq 0.01$), ethyl decanoate and ethyl 9-decenoate (significant at $P \leq 0.001$). Ethyl hexadecanoate was found only in "D". The amount of alcohol free total volatiles

was approximately twice as much in wines inoculated with *Saccharomyces*. Spontaneous fermentation ("A") was most similar to vinification with *Candida* only ("B"). The "A" and "B" samples differed in their fruit esters, ethyl butyrate, ethyl hexanoate and hexyl acetate content, and spontaneous fermentation produced higher level of these constituents. Vinification by *Saccharomyces cerevisiae* was characterised by the presence of longer carbon chain ethyl esters, described in the literature by a soap-like, heavy smell (MIKLÓSY & PÖLÖSNÉ, 1997). However, the average sensory scores of samples did not indicate sensible differences. Total scores on a 25 point scale were as follows: "A": 19.5±0.5; "B": 23.4±1.1; "C": 21.2±3.9; "D": 21.0±0.5.

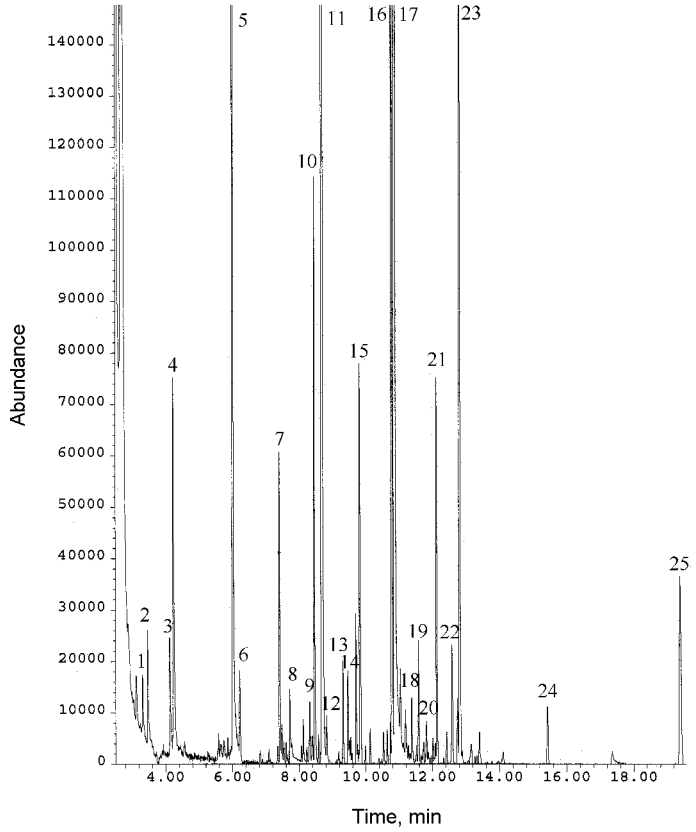

Fig. 1. Total ion chromatogram of Tokaji Aszú fermented with *Saccharomyces cerevisiae* (Sample D), axes: X=time, min, Y=abundance. Peak numbers: 1: ethyl butyrate, 2: ethyl lactate, 3: 1-hexanol, 4: 3-methylbutyl acetate, 5: ethyl hexanoate, 6: hexyl acetate, 7: ethyl heptanoate, 8: 2-phenylethanol 9: caprylic acid, 10: diethyl succinate, 11: ethyl octanoate, 12: decanal, 13: ethyl phenylacetate, 14: phenylethyl acetate, 15: ethyl nonanoate, 16: ethyl 9-decenoate, 17: ethyl decanoate, 18: 3-methylbutyl octanoate, 19: dimethyl naphtalene, 20: ethyl undecanoate, 21: BHT, 22: nerolidol, 23: ethyl dodecanoate, 24: ethyl tetradecanoate, 25: ethyl hexadecanoate

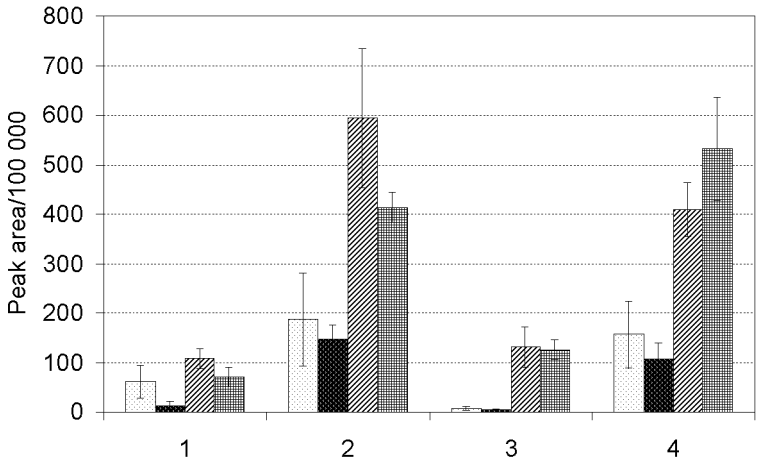

Fig. 2. Effect of different yeasts on some major ester components of Tokaji Aszú wine.
A ⬚: spontaneous fermentation; **B** ■: *Candida stellata* only, **C** ▨: *Candida stellata* + *Saccharomyces cerevisiae*; **D** ▦: *Saccharomyces cerevisiae* only. **1**: ethyl hexanoate; **2**: ethyl octanoate; **3**: ethyl 9-decenoate; **4**: ethyl decanoate

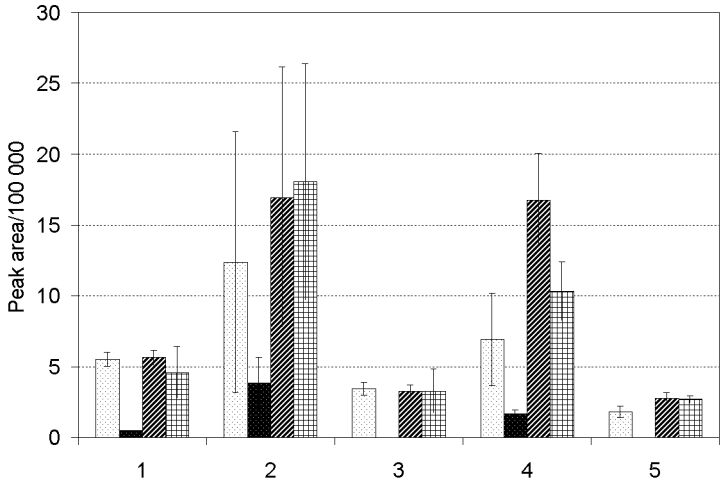

Fig. 3. Effect of different yeasts on some minor ester components of Tokaji Aszú wine
A, B, C, D: as in Fig. 2. **1**: Ethyl butyrate; **2**: 3-methylbutyl acetate; **3**: hexyl acetate; **4**: ethyl heptanoate; **5**: phenylethyl acetate

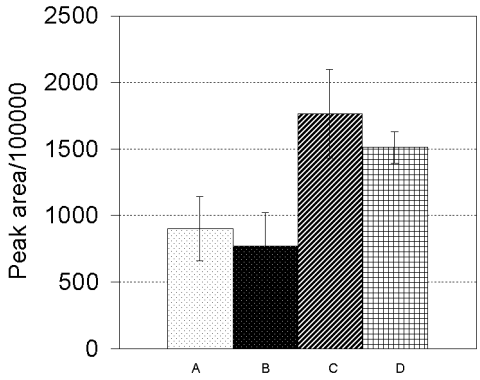

Fig. 4. Effect of different yeasts on alcohol-free total volatile substances of Tokaji Aszú wine.
A, B, C, D: as in Fig. 2

The large standard deviation of "C" was probably due to different reducing sugar content of the replicate fermentations, influencing the taste score.

The main conclusion was that the application of a *Saccharomyces cerevisiae* starter alone would accelerate the fermentation, however this caused only slight change in the aroma profile and raised the total aroma content by producing more esters of longer carbon chain fatty acids. Contribution of *Candida stellata* to the aroma production proved to be weak. Especially the production of longer carbon chain ethyl esters was poor.

2.2. Sulphur compounds

An attempt was made to detect sulphur compounds, which are possibly present in most types of wines. The most frequently detected sulphur compounds, among others 3-methylthio propanol, S-methyl and S-ethyl thioacetate, were searched in total ion chromatograms of wines, based on characteristic fragment ions found in database. Except for sulphur dioxide, no other sulphur compounds were found using different sample preparation techniques (simultaneous steam distillation-extraction with n-pentane and SPME).

The applicability of SPME for the extraction of volatile sulphur compounds was proved by the spiking of diethyl disulphide to Tokaji Aszú in low concentrations. Using selected ion monitoring (ions 122, 66, 94), the detection of 2 $\mu g\ l^{-1}$ diethyl disulphide was possible.

Table 1. Volatile compounds in headspace of a 3-butt Aszú wine from 1993

Peak No.	Compound	Area % mean[a]	Area % st. deviation[a]
1	3-Methyl-butanol	13.85	0.22
2	Ethyl butanoate	0.66	0.02
3	Ethyl lactate	0.56	0.03
4	Furfural	0.33	0.02
5	Ethyl 2-methyl butanoate	0.26	0.03
6	Ethyl 3-methyl butanoate	0.65	0.04
7	Hexanol	0.38	0.03
8	3-Methyl-1-butyl acetate	1.56	0.13
9	Hexanoic acid	0.09	0.004
10	Ethyl hexanoate	4.89	0.44
11	Hexyl acetate	0.17	0.02
12	Ethyl 2-furoate	0.08	0.004
13	Sorbic acid	0.19	0.06
14	Ethyl sorbate	4.65	0.13
15	2-Phenylethanol	1.26	0.15
16	Nerol oxide	0.14	0.01
17	Octanoic acid	0.21	0.03
18	Diethyl succinate	6.63	0.18
19	Ethyl octanoate	23.40	0.75
20	Decanal	0.28	0.02
21	Ethyl phenylacetate	0.37	0.01
22	Phenylethyl acetate	0.29	0.07
23	Vitispirane	9.31	0.28
24	Trimethyl-dihydronaphtalene	5.55	0.42
25	Ethyl 9-decenoate	0.31	0.01
26	Ethyl decanoate	11.78	1.58
27	Dodecanal	0.22	0.03
28	2.7-Dimethyl naphtalene	0.25	0.02
29	Ethyl dodecanoate	0.71	0.34
30	Naphtalene derivative	0.30	0.03
31	Ethyl tetradecanoate	0.27	0.12
	Total - (ethanol+ethyl acetate)	91.21	0.08

[a] mean and standard deviation of 3 replicates

2.3. Bottle ageing of Tokaji Aszú

The aroma profile of bottle aged Tokaji Aszú wines of different butt numbers produced by the same firm was also studied. Table 1 shows the area % composition of a 3-butt Aszú from 1993, while the chromatogram is presented in Fig. 5.

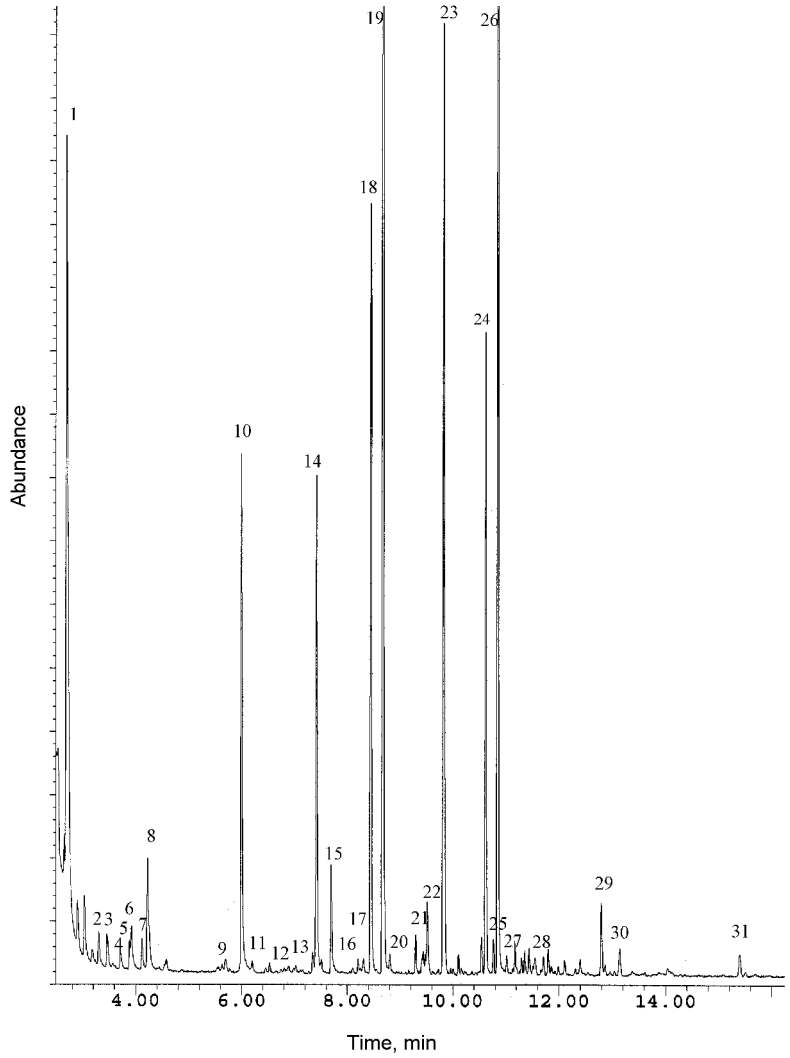

Fig. 5. Total ion chromatogram of the headspace of a 3-butt Tokaji Aszú wine from 1993. Peak numbers: see Table 1

The composition of the wine aroma is surprisingly similar to that of Chardonnay and Semillon white wines studied by LEINO and co-workers (1993) using a dynamic headspace method. The relative proportion of diethyl succinate, vitispirane and trimethyl dihydronaphtalane (TDN) in stored Tokaji wine is approximately by an order of magnitude higher than that in the data of LEINO mentioned above. (Vitispirane and

TDN are nor-isoprenoid volatiles formed from non-volatile precursor compounds of grape.) At the same time, aliphatic esters, among others ethyl octanoate and decanoate are present in Tokaji Aszú in a lower concentration than in young Chardonnay and Semillon white wines.

Further on, the aroma composition of Tokaji Aszú shows an agreement with the results of WALLNER and his group (1999) with Riesling wines.

Certain additives used during processing are also present in chromatograms (sorbic acid in free form and as ethyl sorbate, BHT).

In spite of the fact that the studied wines were bottled in different vintage years from 1989 to 1999, had different butt numbers from 3 to 6, and were stored at Royal Tokaj until year 2000 under unknown conditions, a certain tendency can be discovered in their aroma profile. The concentrations of most ester compounds, namely 3-methyl-butyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate and ethyl dodecanoate decreased with storage, which leads to reduction of the difference in flavour profile caused by yeast strains. Figure 6 displays the trend of some esters as affected by bottle ageing. (Vintage year range from 1989 to 1999 on X axis corresponds to a 12 to 2 years storage time). This figure shows the peak areas of four esters and their linear trend lines as a function of bottling time.

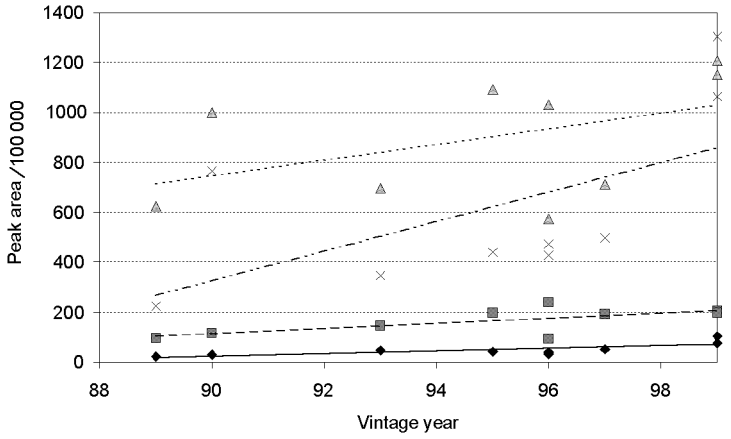

Fig. 6. Relative amount of certain esters of Tokaji Aszú wines from different vintage years, aged in bottles. ◆ : 3-methylbutyl acetate, —— : 3-methylbutyl acetate trend line, ■ : ethyl hexanoate, – – : ethyl hexanoate trend line, ▲ : ethyl octanoate, - - - : ethyl octanoate trend line, X : ethyl decanoate, – · · – : ethyl decanoate trend line

The regression analysis of the data shows a high correlation of vintage time and peak areas of 3-methyl-butyl acetate as well as ethyl dodecanoate at P=0.05% level. Ethyl hexanoate, ethyl decanoate and ethyl octanoate are correlated with storage time at higher than 90% probability level.

Plotting the absolute peak areas versus vintage year, the concentration of vitispirane and trimethyl dihydronaphtalene, both well-known ageing markers (SCHREIER, 1984 and RAPP & VERSINI, 1997) grow. The level of diethyl succinate and 2-phenylethanol showed also a slight increase with time. These trends are statistically not significant due to the different butt numbers and vintage-to-vintage variations.

3. Conclusion

The *Saccharomyces cerevisiae* starter alone accelerated the fermentation but the ethyl esters of higher fatty acids dominated. Contribution of *Candida stellata* to the aroma production proved weak; mainly lower carbon number esters, having a fruity note, evolved, similarly to spontaneous fermentation.

No sulphur compounds except for sulphur dioxide were found in Tokaji Aszú wine, although the applicability of the SPME technique for sulphur compounds was proven by model compounds at low ppb level.

Bottle ageing in Aszú wines of different butt numbers, produced by the same winery was characterised by the decrease of most esters and formation of ageing markers vitispirane and trimethyl dihydronaphtalene as well as elevation of the amount of diethyl succinate and 2-phenylethanol. The difference in flavour profile caused by yeast strains decreases during storage.

SPME is useful in screening of aroma in young and old Tokaji wines as well.

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This work was supported by the Hungarian Ministry of Education, FKFP 0096/1999, and the Hungarian Ministry of Agriculture and Regional Development.

We thank Dr. GY. HAJÓS for bottle-aged wine samples and Dr. F. BOROSS for advice and help.

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