FERMENTATIVE AROMA IN WINES FROM *VITIS VINIFERA* CV. KALECIK KARASI IN RELATION WITH INOCULATION WITH SELECTED DRY YEASTS

ARÔME DE FERMENTATION DANS LES VINS DE *VITIS VINIFERA* CV. KALECIK KARASI EN RELATION AVEC L'INOCULATION DE LEVURES SÈCHES SÉLECTIONNÉES

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Abstract: This study was carried out to investigate the effect of addition of selected *S. cerevisiae* yeasts on cv. Kalecik karası wines. Kalecik karası grown in Middle Anatolia is a native grape variety of *Vitis vinifera*. Inoculation with the selected yeasts produced higher amounts of ethanol compared to spontaneous fermentation and repressed the non-*Saccharomyces* yeasts earlier. The total concentration of flavour compounds increased by using selected wine yeasts. Higher alcohols were abundant compounds and isoamyl alcohol levels varied from 75 to 132 mg/l. The addition of *S. cerevisiae* yeasts produced higher concentrations of 4-vinyl phenol and isoamyl acetate. Wines were also analysed by sensory evaluation. Those obtained with selected yeasts differed from the control wine in triangle test. The most preferred wine was the one produced by commercial *S. cerevisiae* in the ranking test.

Résumé : Cette étude a été menée pour élucider les effets de l'addition de souches sélectionnées de levures *Saccharomyces cerevisiae* sur des vins issus du cépage Kalecik karası. Le Kalecik karası est une variété native de *Vitis vinifera*, implantée en Anatolie moyenne. L'inoculation avec des levures sélectionnées entraine une production plus importante d'éthanol, comparativement à une fermentation spontanée. De plus, les levures n'appartenant pas à l'espèce *Saccharomyces* sont réprimées plus tôt. La concentration totale en composés aromatiques augmente en utilisant des levures sélectionnées. Les alcools supérieurs sont abondants et le niveau d'alcool isoamylique varie de 75 à 132 mg/l. L'addition de levures *S. cerevisiae* entraine une production de plus grandes concentrations de 4-vinyl phénol et d'acétate d'isoamyl. Les vins ont également été analysés par évaluation sensorielle. En test triangulaire, les vins obtenus avec les levures sélectionnées sont différenciés du témoin. En test de rang, le vin préféré est celui produit par la souche commerciale de *S. cerevisiae*.

Key words: winemaking, yeast inoculation, volatile compounds, Kalecik karası cv.

Mots-clés : vinification, levures sélectionnées, composés volatiles, Kalecik karası cv.

INTRODUCTION

The fermentation of grape juice into wine is either conducted by spontaneous (natural) fermentation with the flora present on the surface of grapes or by the inoculation of juice with a known culture of *Saccharomyces* (S.) *cerevisiae* (BOULTON *et al.*, 1996). Spontaneous fermentation is practiced in many wineries using traditional methods for wine production. In the traditional practice, the fermentation has been described to be initiated with weakly fermenting non-*Saccharomyces* yeasts, mainly *Kloeckera apiculata*/*Hanseniaspora uvarum* and *Candida* spp. followed by replacement of more ethanol tolerant yeasts of *S. cerevisiae* in the course of fermentation (REED and NAGODAWITHANA, 1988). Inoculation of the grape juice with selected cultures of *S. cerevisiae* is also widely used for improving wine production by winemakers. These cultures are chosen either from local *S. cerevisiae* strains or commercially available wine yeasts, therefore a better quality of product is usually obtained than the wine made by spontaneous fermentation (FLEET and HEARD, 1993; REGODON *et al.*, 1997).

The production of flavour compounds during alcoholic fermentation is closely related to yeasts involved. These components formed by yeast metabolism affect the composition and quality of the wine (CABRERA *et al.*, 1988; RAPP, 1998; RIBEREAU-GAYON *et al.*, 2000). Flavour compounds are responsible for the typical odor and taste of wine. They are mainly higher alcohols, esters, fatty acids, carbonyl compounds and volatile phenols (RAPP and MANDERY, 1986).

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Kalecik karası is a native grape variety of *Vitis vinifera*, grown in the Ankara district of the Middle Anatolia Region of Turkey. This variety produces one of the best red wines of Turkey. However, the flavour compounds of cv. Kalecik karası wines have not yet been reported. Thus, the aim of this study was to investigate the differences in alcoholic fermentation and flavour compounds between wines produced with spontaneous fermentation and wines made by inoculation with selected *S. cerevisiae* yeasts.

MATERIALS AND METHODS

I- YEASTS

The commercial *S. cerevisiae* yeast used was Fermirouge 7303 (Gist Brocades, France). The indigenous *S. cerevisiae* was isolated during the fermentation of cv. Kalecik karası must in 1998 vintage.

II- FERMENTATION

Healthy grapes (about 125 kg) of cv. *Vitis vinifera* Kalecik karası were obtained from the vineyard of Kavaklıdere Winery (Akyurt, Ankara) and transported to the Pilot Winery of Department of Food Engineering, University of Çukurova, Adana.

The grapes were destemmed and crushed and then, 35 mg/kg of sulphur dioxide was added. Crushed grapes were separated into three parts to carry out the following fermentations: a) spontaneous fermentation (without inoculation), b) fermentation with indigenous *S. cerevisiae* and c) fermentation with commercial *S. cerevisiae*.

The inoculation for indigenous *S. cerevisiae* was performed with a 2 days old culture propagated on an orbital shaker according to ERTEN (1997). The commercial yeast was suspended in sterile warm water at 35 °C according to the producer's instructions. The inoculum level for both yeasts was approximately $5x10^6$ cells/kg.

Fermentations were conducted in 50 l stainless steel tanks containing 40 kg of crushed grapes at 20-25 °C under fermentation locks. The maceration time was 6 days. Fermentation was followed by drop in sugar content of must. After the fermentation, the young wine was pressed by a manual press and left for malolactic fermentation at 20 °C. Malolactic fermentation was monitored by paper chromatography. After the malolactic fermentation was completed, wines were racked and 75 mg/L of sulphur dioxide was added. They were racked two times during the maturation and after each racking, 50 mg/L of sulphur dioxide was added. Wines were evaluated by sensory analysis after bottling.

III- ENUMERATION OF THE YEAST POPULATION

Daily samples taken during alcoholic fermentation were diluted as necessary and then spread on malt extract agar (Difco) and lysine agar (Difco) for enumeration of total yeasts and total non-*Saccharomyces* yeasts, respectively (FLEET and HEARD, 1993; CAMPBELL, 1988).

IV- GENERAL WINE ANALYSIS

Ethanol, total acidity, pH, volatile acidity, acetaldehyde and reducing sugar were analysed (ANON., 1990; OUGH and AMERINE, 1988).

V- FLAVOUR COMPOUNDS ANALYSIS

Flavour compounds of wines were extracted in the Biotechnology Laboratory of the Department of Food Engineering of the University of Cukurova, Adana, Turkey. Identification and quantification of flavour compounds were performed in the Aroma Laboratory of INRA in Montpellier (France). Wine samples were taken after the alcoholic fermentation, before malolactic fermentation started. Extraction of flavour compounds was performed according to previous studies (BLANCH *et al.*, 1991; SCHNEIDER, *et al.*, 1998). Before extraction, 10 µl of 4-nonanol (3.4 mg/l in methanol) as internal standard and then 40 ml of dichloromethane were pipetted into 500 mL flask containing 100 mL of wine. The content was magnetically stirred for 30 min under nitrogen gas at 4-5 °C. Then, the mixture was centrifuged at 9000xg for 15 min at 0 °C. The organic phase was recovered, filtered through glass wool with anhydrous sodium sulfate and concentrated to a volume of 1 mL with a Vigreux distilation column. The process was performed in duplicate. The samples were stored at –18 °C until GC-FID and GC-MS analyses.

Flavour compounds were measured by a Varian 3300 GC with FID at 250 °C and a fused capillary column coated with DB-Wax (30 m x 0.32 mm i.d., 0.5 mm film thickness, JW, Folsom, CA, USA). The carrier gas was hydrogen at the flow rate of 2 ml/min. The injection mode was on-column. On-column injector temperature was programmed from 20 to 250 °C at 180 °C/min. Oven temperature was kept at 60 °C for 3 min, and then increased to 220 °C at 2 °C/min. It was then risen from 220 °C to 245 °C at 3 °C/min and kept at 245 °C for 20 min. One microliter of sample was injected for each analysis.

The flavour compounds were identified by GC-MS. A Hewlett-Packard 5890 Series II chromatograph was used with the above mentioned column. The injection mode was on-column. Temperature programmes of the injector and oven were as described above. The flow

rate of the helium gas as carrier was 1.5 ml/min. A Hewlett-Packard 5989A mass spectrometer equipped with a quadrupole detector was used for electron impact (EI). The source temperature was 250 °C. EI was recorded at 70 eV in the range m/z 29-350 at 1 s intervals.

Flavour compounds were identified by comparing linear retention index and by electronic mass spectrum matching with published data or with authentic compounds. They were, then, quantified by the internal standard method (4-nonanol as internal standard) using the FID response factor previously measured by standard flavours and expressed by the means of duplicate analytical assays.

VI- SENSORY AND STATISTICAL ANALYSIS

Wines were evaluated by triangle and ranking tests. Flavour compounds were compared by varience analysis and «Least Significant Difference» analysis (AMERINE and ROESSLER, 1976). The detailed statistical tables were used for triangle test and ranking test was analysed by Friedman test (AMERINE and ROESSLER, 1976; BARILLERE and BENARD, 1986).

RESULTS AND DISCUSSION

I- EFFECT ON THE FERMENTATION RATE AND YEAST GROWTH

Addition of selected yeasts of *S. cerevisiae* showed a marked effect on the fermentation rate as given in figure 1. Inoculation with *S. cerevisiae* yeasts led to faster decrease in the utilisation of sugars and both fermentations were completed on day 4. Spontaneous fermentation brought about slower fermentation rate compared to inoculated fermentations. In the present study, fermentation rate increased with the addition of yeast as shown in figure 1 in accord with previous reports (HEARD and FLEET, 1986; FLEET and HEARD, 1993).

The growth of total yeasts and total non-*Saccharomyces* spp. yeasts are shown in figure 2. Addition of indigenous and commercial *S. cerevisiae* increased the total counts with maximum numbers of about 10.8 log cfu/mL, then followed a short stationary phase. The numbers in fermentations conducted with inoculation of selected yeasts decreased to 6.2-6.5 log cfu/mL at the end of fermentation. The non-*Saccharomyces* yeasts exhibited slight increase during alcoholic fermentation and were not isolated after day 2 in the fermentations inoculated with indigenous and commercial *S. cerevisiae*. These yeasts died off by day 4 in the spontaneously fermented wine. It is clear that the non-*Saccharomyces* species disappeared as *S. cerevisiae* exerted its dominance.

Natural grape juice fermentation involves different yeast populations. The non-*Saccharomyces* yeasts grow during the early stage of fermentation. In contrary to the general assumption (AMERINE *et al.*, 1980; FARKAS, 1988), the non-*Saccharomyces* spp. can survive during the fermentation and are not suppressed by the inoculation of *S. cerevisiae* (HEARD and FLEET, 1986; FLEET and HEARD, 1993). In the present paper, inoculation of cv. Kalecik karası musts with indigenous and commercial yeasts repressed the non-*Saccharomyces* spp. by day 2 (figure 2) corfirming the results of HEARD and FLEET (1988), CIANI (1997) and EGLI *et al.* (1998).

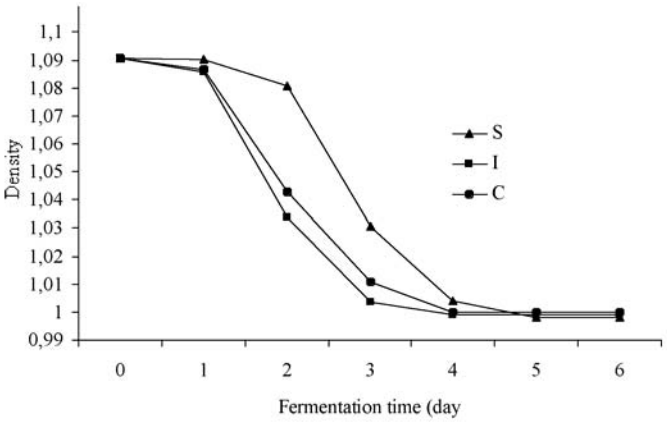

Fig. 1 - The evolution of density of cv. Kalecik karası musts
S: Spontaneously fermented wine, I: Wine inoculated with indigenous yeast, C: Wine inoculated with commercial yeast

Évolution de densité des moûts Kalecik karası

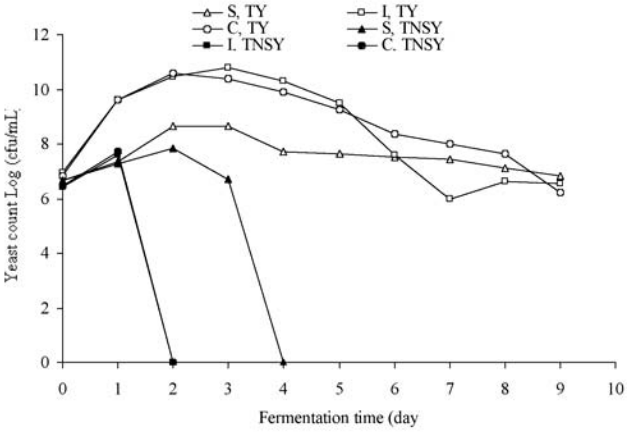

Fig. 2 - Dévelopment in the total yeast and the total non-*Saccharomyces* yeasts in cv. Kalecik karası musts during the fermentation
S,TY: Total yeast of spontaneously fermented wine, I,TY: Total yeast of wine inoculated with indigenous yeast, C,TY: Total yeast of wine inoculated with commercial yeast. S,TNS: Total non-*Saccharomyces* yeasts of spontaneously fermented wine, I,TNSY: Total non-*Saccharomyces* yeasts of wine inoculated with indigenous yeast, C,TNSY: Total non-*Saccharomyces* yeasts of wine inoculated with commercial yeast

Développement des levures totales et des levures non-saccharomyces totales de Kalecik karasi au cours de la fermentation

Table I - Chemical analysis of cv. Kalecik karası wines

Analyses chimiques des vins de Kalecik karası

	S	I	C
Ethanol (%, v/v)	11.56	11.85	12.15
Total acidity (meq/L)	77	81	78
pH	3.5	3.5	3.6
Volatile acidity (meq/L)	4	4	4
Acetaldehyde (mg/L)	20	32	24
Reducing sugar (g/L)	0.75	1.34	0.82

S: Spontaneously fermented Wine I: Wine produced with inoculation of indigenous yeast,
C: Wine produced with inoculation of commercial yeast

II- GENERAL WINE COMPOSITION

Table I lists the chemical analysis of cv. Kalecik karası wines. Commercial *S. cerevisiae* formed 12.15 % (v/v) of ethanol. The volatile acidity of the wines was 4 meq/L and the reducing sugar level less than 1.34 g/L.

III- WINE FLAVOUR COMPOUNDS

The concentrations of flavour compounds formed in cv. Kalecik karası wines were compared in table II. Addition of selected yeasts increased total concentrations of flavour compounds in the following order: wine produced by addition of commercial *S. cerevisiae* > wine produced by inoculation of indigenous *S. cerevisiae* > wine produced by spontaneous fermentation.

Analyses of variance and least significant difference revealed differences in individual flavour compounds. Addition of indigenous and commercial *S. cerevisiae* yeasts increased the total concentration of higher alcohols formed in cv. Kalecik karası wines which were 146.6 mg/L and 171 mg/L respectively. Total amount of higher alcohols in spontaneously fermented wine was found 105 mg/L. Inoculation with commercial yeast showed higher amounts of isoamyl alcohol, isobutanol and heptanol, whereas higher concentrations of 2-phenyl ethanol, 1-butanol and benzyl alcohol were obtained in the wine inoculated with indigenous *S. cerevisiae*. The wine fermented by spontaneous fermentations, however, gave greater amount of 2,3-butandiol. RAPP and VERSINI (1991) state that higher alcohols are the main volatile compounds in quantitative terms, contributing desirable aroma to wines at total concentrations below 300 mg/L. These alcohols give detrimental effect when the total amounts exceed 400 mg/L. In the present study, only isoamyl alcohol levels exceeded the threshold value of 60 mg/L given in wine by SIMPSON (1979), but the concentrations were much lower than the highest threshold value of 180 mg/L stated by RIBEREAU-GAYON (1978). 2-Phenyl ethanol was the second abundant compound but concentrations were at subthreshold levels in the wine given by AMERINE AND ROESSLER (1976).

There was a higher production of esters in the wine made by the inoculation of the commercial yeast due mainly to formation of ethyl lactate with the highest concentration in contrast to the other two wines as shown in Table II. When ethyl lactate was not included, wines had lower total amounts of esters (less than 1.7 mg/L). However, the concentrations of isoamyl acetate and 2-phenylethyl acetate of acetate esters, ethyl hexanoate, ethyl octanoate and ethyl decanoate of fatty acid esters and phenylethyl lactate of organic acid esters were significantly increased by inoculation with indigenous and commercial *S. cerevisiae*. Differences in ester formation is closely related to yeast species and within yeast species to strain involved in alcoholic fermentation (HERRAIZ *et al.*, 1990; MATEO *et al.*, 1991; MATEO *et al.*, 1992). Ethyl esters of acetates and medium chain fatty acids are important sensory contributers to wine, giving fruity and sweetish flavour at concentrations exceeding their flavour threshold levels (RAPP and MANDERY, 1986; SIMPSON, 1979; LOPEZ *et al.*, 1999). In the present study, inoculation with the commercial *S. cerevisiae* yeast increased the total concentration of esters. With the exception of isoamyl acetate, the amounts of esters in all wines were below their threshold levels given by ETIEVANT (1991). The concentrations of isoamyl acetate in the wines obtained by the addition of *S. cerevisiae* strains were close to its flavour threshold level of 1 mg/L in wine (ETIEVANT, 1991).

Addition of *S. cerevisiae* yeasts produced a marked effect on fatty acids with about 2 fold increase in the contents compared to the wine made spontaneously. Butyric acid, octanoic acid and hexanoic acid were the main compounds formed together with nonanoic acid showing much higher levels with inoculation of selected yeasts. As can be seen from table II, fatty acid concentrations were much less than their threshold levels (ETIEVANT, 1991) and their contribution to cv. Kalecik karası wine flavour may be excluded in agreement with CABAROGLU *et al.* (1997). With regard to phenols,

Table II - Influence of yeast inoculation on volatile compounds of cv. Kalecik karası wines

Incidence de l'inoculation de levure sur les teneurs en composés volatils des vins de Kalecik karası

			Concentration (µg/L)			
Compounds	RI[v]	ID[y]	S	I	C	Sig.[z]
Higher alcohols						
Isobutanol	1096	A	12423[a]	7946[b]	22403[c]	***
1-Butanol	1120	B	370[a]	409[b]	177[c]	***
Isoamyl alcohol	1209	A	75025[a]	115655[b]	132020[c]	***
Pentanol	1249	B	52[a]	46[b]	51[a]	*
Heptanol	-	C	13[a]	31[b]	44[c]	***
2,3-Butandiol	1579	B	3105[a]	1331[b]	1899[c]	***
Benzyl alcohol	1865	A	0.58[a]	316[b]	208[c]	***
2-Phenyl ethanol	1902	A	13967[a]	20900[b]	14175[a]	***
Total of higher alcohols			104956	146634	170977	
Esters						
Isoamyl acetate	1125	A	468[a]	812[b]	834[b]	***
Ethyl hexanoate	1230	A	114[a]	313[b]	184[c]	***
Hexyl acetate	1307	B	9	7	7	ns
Ethyl lactate	1353	A	3078[a]	2201[b]	13838[c]	**
Etyhl octanoate	1430	A	237[a]	180[b]	234[a]	***
Ethyl decanoate	1635	A	101[a]	237[b]	278[c]	**
2-Phenyl ethyl acetate	1820	B	12[a]	46[b]	126[c]	***
Ethyl dodecanoate	-	C	10	------	----	***
Ethyl phenyl lactate	-	C	2[a]	3[b]	14[c]	**
Total of esters			4031	3799	15515	
Volatile acids						
Propanoic acid	-	C	-----[a]	17[b]	11[ab]	***
Butyric acid	1610	B	402[a]	2275[b]	2308[b]	***
Hexanoic acid	1838	B	145[a]	921[b]	989[c]	**
Octanoic acid	2060	B	1053[a]	1640[b]	1464[c]	**
Nonanoic acid	-	C	12[a]	82[b]	248[c]	***
Decanoic acid	2357	B	437[a]	403[b]	276[c]	**
Dodecanoic acid	2499	B	47[a]	41[b]	48[a]	*
Tetradecanoic acid	2692	B	50[a]	26[b]	110[c]	**
Octadecanoic acid	-	C	416[a]	78[b]	422[a]	***
Total of volatile acids			2562	5483	5876	
Phenols						
4-Vinyl guaiacol	2177	A	--- [a]	5[ab]	9[b]	***
4-Vinyl phenol	2378	A	----[a]	168[b]	692[c]	***
Total of phenols				173	701	
Carbonyl Compounds						
Acetoin	1312	B	789[a]	226[b]	367[c]	***
Total of carbonyl compounds			789	226	367	
Total			112338	156315	193436	

x: The data are mean values of duplicates (maximum SD: ±10); v: RI: Linear retention index calculated on DB WAX capillary column; y: ID: Identification, A: GC retention and MS data in agreement with that of authentic standards, B: GC retention and MS data in agreement with spectra found in the library, C: tentatively identified by MS matching with the library spectra only; z: Sig.: significance at which means differ as shown by analysis of variance: *, **, *** denote significance at $p<0.05$, $p<0.01$, $p<0.001$ respectively.; S: spontaneously fermented wine, I: wine produced by indigenous *S. cerevisiae*, C: wine produced by commercial *S. cerevisiae*.

concentrations of 4-vinyl phenol in wines produced by selected wine yeasts were found higher than its threshold value in model solution given by CHATONNET *et al.* (1993).

IV- SENSORY ANALYSIS

A triangle taste was performed to wines with a spontaneously fermented wine used as the control wine. Five out of the six panelist were able to distinguish the sample obtained by the addition of indigenous *S. cerevisiae* from the control wine at $p<0.05$. All judges indicated that the wine made with the inoculation of commercial *S. cerevisiae* differed from the control wine at $p<0.01$. In the ranking test, the wine made by the addition of commercial *S. cerevisiae* was found the most preferred sample at $p<0.05$ compared to the other two wines.

CONCLUSIONS

This study on cv. Kalecik karası wines obtained with spontaneous fermentation and with inoculated *S. cerevisiae* yeasts showed they were significantly differed in chemical and sensory analyses. Non-Saccharomyces yeasts died off earlier in fermentations performed with the addition of the wine yeasts. All juice samples were fermented to dryness. Higher amounts of ethanol were formed by the use of selected *S. cerevisiae* yeasts. These wine yeasts produced higher amounts of volatile compounds, especially with isoamyl alcohol, 2-phenyl ethanol, isoamyl acetate, 4-vinyl phenol. Wine produced by commercial yeast was the most preferred wine.

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