

# Contribution by *Saccharomyces cerevisiae* Yeasts to Fermentation and Flavour Compounds in Wines from cv. Kalecik karası Grape

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## ABSTRACT

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The effect of indigenous and commercial *S. cerevisiae* yeasts on fermentation and flavour compounds of wines was examined in pasteurised grape juice. The flavour compounds were analysed and identified by GC-FID and GC-MS, respectively and in general, the amounts of these volatiles were increased by the use of both indigenous and commercial yeasts. The levels of isoamyl alcohol, isoamyl acetate, ethyl octanoate and ethyl deconoate exceeded flavour thresholds. All grape juices were fermented to dryness. Selected yeasts produced higher ethanol concentrations compared to spontaneous fermentations.

**Key words:** cv. Kalecik karası, fermentation, flavour compounds, *Saccharomyces cerevisiae*, wine, yeast.

## INTRODUCTION

Wine fermentation has traditionally depended on the activity and growth of indigenous yeasts originating from grapes and winery equipment (i.e. natural or spontaneous fermentation)<sup>30</sup>. In enology selected indigenous local, or commercial *Saccharomyces cerevisiae* strains, can be used to obtain better quality wine than with only natural fermentation<sup>7,30</sup>. Inoculation of grape must with selected yeasts offers better control of alcoholic fermentation and of desirable sensory properties<sup>13,37</sup>.

In addition to the main products of ethanol and carbon dioxide, yeasts produce a wide range of flavour compounds during the alcoholic fermentation of grape must. Higher alcohols, esters and acids are quantitatively abundant in wine flavours<sup>19,25</sup>. Previous studies have shown that the yeasts, which induce the fermentative flavour, are responsible for the great differences in the chemical and sensory properties of wines<sup>5,20,21,24,28,36</sup>.

Kalecik karası is a native Turkish grape variety of *Vitis vinifera*. It is a major black grape variety of the Ankara district and is used for the production of good quality red Kalecik karası wine<sup>1,18</sup>. The aim of this study was to investigate differences in fermentation progress and flavour production, using indigenous and commercial *S. cerevisiae* yeasts, in pasteurised cv. Kalecik karası grape juice, together with spontaneous fermentations for comparison.

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## MATERIALS AND METHODS

### Yeasts

The yeast strain referred to as indigenous *S. cerevisiae* in this text was selected according to its technological properties from the wine yeasts isolated and identified as *S. cerevisiae* during cv. Kalecik karası wine fermentations in 1998. The identification of the yeasts was carried out according to API 32C Auxanogramm and API Lab Software (bioMerieux API Systems, Marcy-l'Etoile, France) and Barnett *et al.*<sup>7</sup>. The commercial culture of *S. cerevisiae* (Fermirouge 7303-Gist Brocades) was obtained from INRA, France.

### Medium

The cv. Kalecik karası grape juice was used. Grape juice was prepared by the thermovinification method to promote the colour extraction<sup>3</sup>. Healthy grapes (200 kg) from the vineyard of the Kavaklıdere Winery (Akyurt, Ankara) were transported to the pilot winery of the Department of Food Engineering, University of Çukurova, Adana. They were de-stemmed and crushed. The crushed grapes were held at 65°C for 15 min to allow colour release and then cooled immediately. Free run grape juice was removed and the pomace was manually pressed. Free and pressed grape juices were combined and pasteurised at 75°C for 15 min.

### Inocula

The inoculation, using the selected indigenous *S. cerevisiae*, was performed with a 2 day old culture, propagated in sterile grape juice on an orbital shaker according to Erten<sup>15</sup>. The commercial yeast culture was suspended in sterile warm water at 35°C according to the manufacturer's instruction. The pitching rate for both indigenous and commercial yeasts was 5×10<sup>6</sup> cells/mL. For comparison, a *pie de cuve* type of inoculation<sup>37</sup> was prepared to determine the effect of natural flora. For this, healthy grapes (3 kg) of cv. Kalecik karası were crushed in a clean nylon bag and grape juice was added to the fermentation vessel. The yeast cell number was 8×10<sup>5</sup> cells/mL.

### Fermentation conditions

The fermentations were performed, in duplicate, in 20 litre chemically sterilized glass vessels containing 16 litres of pasteurised grape juice. Fermentation vessels were closed using chemically sterilised fermentation locks. Fer-

mentation was monitored by measuring the concentration of reducing sugars in the fermented grape juice. After the alcoholic fermentation was complete, the wines were racked and inoculated with a malolactic culture, consisting of the lees of the wine which had already completed a malolactic fermentation. Fermentation was judged by the degradation of malic acid using paper chromatography. After the malolactic fermentation was complete, wines were racked and 75 mg/litre of sulphur dioxide (i.e. 1.5 mL/litre from a 5% solution of sulphur dioxide) was added. The wines were stored at 15°C for maturation. They were racked two times during the maturation and after each racking, 50 mg/litre of sulphur dioxide was added. Sensory analysis was performed after bottling.

### Enumeration of yeast population

Daily samples were taken during the alcoholic fermentation and the number of yeast counted using methylene blue staining and a Thoma counting chamber<sup>6</sup>.

### General wine analysis

Density, ethanol, extract, total acidity, pH, volatile acidity, acetaldehyde, reducing sugars, total and free SO<sub>2</sub>, total phenolics (optical density at 280 nm), tannins, anthocyanins, colour intensity and colour tint were analysed<sup>4,27</sup>.

### Analysis of flavour compounds

Extraction of flavour compounds from the wines was conducted in the biotechnology laboratory of the Department of Food Engineering, University of Cukurova, Adana, Turkey and the identification and quantification of flavour compounds was performed in the aroma laboratory of INRA, Montpellier, France.

Wine samples were analysed after the completion of the alcoholic fermentation. Extraction of flavour compounds was performed according to Blanch *et al.*<sup>9</sup> and Schneider *et al.*<sup>34</sup>.

Before extraction, 10 µL of 4-nonanol (34 µg/litre) as internal standard and 40 mL of dichloromethane were pipetted into a 500 mL flask containing 100 mL of wine. The content was stirred using a magnet, for 30 min under nitrogen gas, at 4–5°C. Then, the mixture was centrifuged at 9000 g for 15 min at 0°C. The organic phase was recov-

ered, filtered through glass wool with anhydrous sodium sulfate and concentrated to a volume of 1 mL with a Vigreux distillation column. The process was performed in duplicate. The samples were stored at –18°C until analysed by GC-FID and GC-MS.

Flavour compounds were measured using a Varian 3300 GC, with FID at 250°C, and a fused capillary column coated with DB-Wax (30 m × 0.32 mm i.d., 0.5 µm film thickness, JW, Folsom, CA, USA). The carrier gas was hydrogen at a flow rate of 2 mL/min. The on-column injector temperature was programmed from 20 to 250°C at 180°C/min.

The oven temperature was kept at 60°C for 3 min, and then increased to 220°C at 2°C/min. Then it was allowed to rise from 220°C to 245°C at 3°C/min and kept at 245°C for 20 min. One microlitre of sample was injected for each analysis. The injection mode was on-column.

The flavour compounds were identified by GC-MS. A Hewlett-Packard 5890 Series II chromatograph was used with the aforementioned column. The injection mode was on-column. Temperature programs of the injector and oven were as described above. The flow rate of the helium gas 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 the linear retention index and electronic mass spectrum matching with published data or with authentic compounds. These were quantified by the internal standard method (4-nonanol as internal standard) using the FID response factor previously measured by standard flavours and expressed by means of duplicate analytical assays.

### Chemicals

High-purity chemicals were obtained from Merck, Fluka and Sigma (France).

### Sensory analysis

Wines were evaluated by using the triangle test and a group of 6 panelists, due to difficulty in finding experts in sensory analysis. The panel was organized according to the principles of sensory assessment described by Amerine & Roessler<sup>2</sup>.

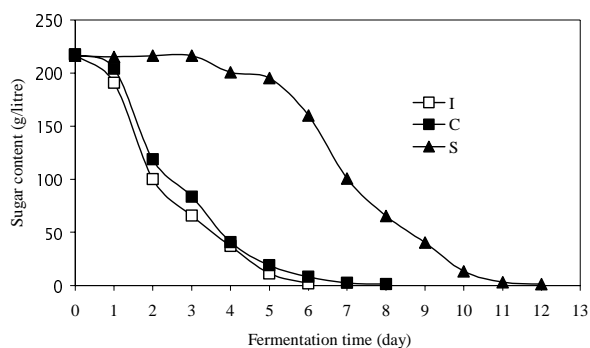


FIG. 1. Sugar content during the fermentation of cv. Kalecik karasi grape juice. S: Spontaneously fermented wine (▲); I: wine fermented with indigenous *S. cerevisiae* (□); C: wine fermented with commercial *S. cerevisiae* (■).

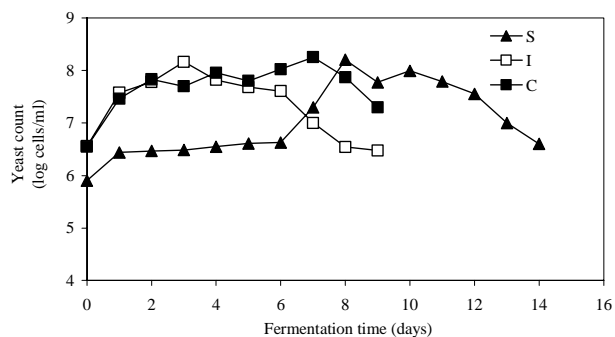


FIG. 2. Yeast count during the fermentation of cv. Kalecik karasi grape juice. S: Spontaneous fermentation (▲); I: indigenous *S. cerevisiae* (□); C: commercial *S. cerevisiae* (■).

## Statistical analysis

Flavour compounds were compared by variance analysis and least significant difference analysis<sup>2</sup>. The resulting scores of sensory analysis were compared using detailed statistical tables<sup>2</sup>.

## RESULTS AND DISCUSSION

The following fermentations were carried out to explain the effect of adding selected yeasts on cv. Kalecik karası wines; i) spontaneous (*Pied de cuve*) fermentation ii) fermentation with indigenous *S. cerevisiae* and iii) fermentation with commercial *S. cerevisiae*. The indigenous yeast was selected according to the technological characteristics of fermentation rate, ethanol and volatile acid formation, resistance to sulphur dioxide, killer phenotype and high temperature growth (results not shown).

### Fermentation rate

Fig. 1 shows the consumption of the reducing sugars during the fermentation. The fermentation rate of the indigenous *S. cerevisiae* was faster than that of the commercial yeast culture. The concentration of residual sugar was below 2 g/litre on day 6 for the indigenous yeast, and on day 8 for the commercial *S. cerevisiae* yeast. Spontaneous fermentation exhibited a slow fermentation rate and reached dryness on day 12. Several researchers<sup>13,30,31</sup> have discussed the increased use of indigenous (local) and commercial *S. cerevisiae* strains for wine making. Fermentations performed with indigenous and commercial wine yeasts were completed faster than using the spontaneous process and this is in agreement with Fleet and Heard<sup>17</sup>.

### Yeast growth

The growth of viable yeast during the fermentation is shown in Fig. 2. The change in the yeast population of the indigenous and commercial yeasts was more rapid than in the spontaneous fermentation. Fermentations reached the

highest counts (approximately 8.2 log cell/mL) during the course of the fermentation. At the end of fermentation viable counts ranged from 6.4 to 7.3 log cell/mL. Fleet and Heard<sup>17</sup> reported that the viable numbers of yeasts in grape juice rises from 4–6 log cfu/mL to 8–9 log cfu/mL during a typical fermentation.

### General wine composition

The composition of cv. Kalecik karası wines is given in Table I. The commercial strain of *S. cerevisiae* produced 12.75% (v/v) of ethanol. Wines were fermented to dryness (i.e. less than 1.75 g/l of reducing sugar). Volatile acidity, as acetic acid, was less than 0.36 g/litre. Acetaldehyde ranged from 17–44 mg/litre. The general composition of wines was in accordance with previous studies carried out on Turkish wines<sup>12,14,33</sup>.

### Production of flavour compounds

Flavour compounds play an important role in the typical odour and taste of wine. Some constituents are present in certain grapes such as Muscat, whereas others are formed during maturation. However, the main producer of flavour components during alcoholic fermentation is the yeast. These flavour components are composed of mainly higher alcohols and esters, followed by fatty acids, carbonyl compounds, sulphur compounds and phenols<sup>23,27</sup>. The levels of volatile compounds in the present study are given in Table II. Spontaneous fermentation, indigenous and commercial yeasts produced 126.4, 162.3 and 191.3 mg/litre of volatiles, respectively in the wines.

Among the higher alcohols, isoamyl alcohol, 2-phenyl ethanol, isobutanol and 2,3-butandiol were the major compounds in the cv. Kalecik karası wines (Table II) and similar results were observed in other studies<sup>8,11,22</sup>. With the exception of isoamyl alcohol, the concentrations were much lower than their flavour threshold values<sup>32,35</sup>. The total amounts of higher alcohols in this study ranged from 113.5 to 162.8 mg/litre; Rapp and Versini<sup>29</sup> reported detrimental effects with the levels exceeding 400 mg/litre.

With regard to esters, isoamyl acetate and ethyl lactate were the most abundant compounds in cv. Kalecik karası wines. Esters are important contributors to the fruity odour in wine<sup>10, 16</sup>. In this study only the concentration of isoamyl acetate, which imparts banana and fruity flavour, exceeded the 1 mg/litre threshold value reported by Simpson<sup>35</sup>, however 16 mg/litre levels have been reported in wines<sup>17</sup>. Amounts of ethyl octanoate and ethyl decanoate produced by the indigenous and commercial *S. cerevisiae* yeasts were higher than the threshold values given by Etiévant<sup>16</sup> but lower than those reported by Simpson<sup>35</sup>.

The indigenous and commercial yeast strains gave increased fatty acid levels; octanoic, hexanoic, decanoic and butyric acids were major compounds in the wines. However, their threshold values did not exceed the levels described in the literature<sup>16,35</sup>.

### Sensory evaluation

Wines were evaluated using a triangle test and 6 trained judges. The wine produced from the spontaneous fermentation was used as the control. Wines, made with the indigenous and commercial yeast strains, were compared

TABLE I. General composition of cv. Kalecik karası wines<sup>1</sup>.

	S	I	C
Density (20°C)	0.9916	0.9917	0.9919
Ethanol (% , v/v)	11.82	12.25	12.75
Extract (g/L)	24.5	21.9	22.12
Total acidity (g/litre as tartaric acid)	4.93	4.97	4.69
pH	3.6	3.69	3.65
Volatile acidity (g/litre as acetic acid)	0.36	0.3	0.3
Acetaldehyde (mg/litre)	44	17	23
Reducing sugar (g/litre)	1.75	1.34	1.26
Free SO <sub>2</sub> (mg/litre)	9	9	8
Total SO <sub>2</sub> (mg/litre)	52	53	65
Total phenolics (280 index)	38	38	40
Anthocyanins (mg/litre)	160	188	169
Tannins (g/litre)	2.2	2.6	1.85
Colour intensity	0.518	0.422	0.627
Colour tint	0.750	1.028	0.699

<sup>1</sup> The data are mean values of duplicate experiments and SD values were below ±4%. S: Spontaneous Wine, I: Wine produced with indigenous *S. cerevisiae*, C: Wine produced with commercial *S. cerevisiae*.

to the control wine. Five out of 6 panelists indicated that there was a significant difference between the control wine and the wine produced with the indigenous yeast ( $p < 0.05$ ). A similar result was obtained between the control wine and the wine made with commercial yeast ( $p < 0.05$ ).

## CONCLUSIONS

According to the results obtained, indigenous and commercial *S. cerevisiae* yeast strains showed greater fermentation power than spontaneous fermentation. Higher amounts of ethanol were obtained by using these yeasts. By the use of selected yeast strains, different concentrations of flavour compounds were increased during fermentations. Isoamyl alcohol, isoamyl acetate followed by ethyl octanoate and ethyl deconoate were formed at their flavour thresholds.

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## REFERENCES

1. Akman, A. and Yazıcıoğlu T., Şarap Kimyası ve Teknolojisi, Ankara Üniversitesi, Ziraat Fakültesi Yayınları, yayın no: 160, Ankara, 1960.
2. Amerine, M.A. and Roessler E.B., Wines: Their Sensory Evaluation, W. H. Freeman and Co. San Francisco, 1976.
3. Amerine, M.A., Berg, H.W., Kunkee, R.E., Ough, C.S., Singleton, V.L. and Webb, A.D., The Technology of Wine Making, 4<sup>th</sup> edn., Avi Publishing Com. Inc., Westport, Connecticut, 1980.
4. Anonymous, Recueil des Methods International d'Analyses des Vins et des Mouts, Office International de la Vigne et du Vin, Paris, 1990.

TABLE II. The production of flavour compounds by selected yeasts in cv. Kalecik karası wine<sup>1</sup>.

Compounds	RI	Iden.	Concentration (µg/l)			Sig.
			S	I	C	
<b>Higher alcohols</b>						
Isobutanol	1096	A	7341 <sup>a</sup>	3931 <sup>b</sup>	6382 <sup>c</sup>	***
1 butanol	1120	B	310 <sup>a</sup>	390 <sup>b</sup>	288 <sup>c</sup>	**
Isoamyl alcohol	1209	A	89368 <sup>a</sup>	117996 <sup>b</sup>	133440 <sup>c</sup>	***
Pentanol	1249	B	37 <sup>a</sup>	8 <sup>b</sup>	62 <sup>c</sup>	***
2,3-Butanediol	1579	B	2752 <sup>a</sup>	741 <sup>b</sup>	3288 <sup>c</sup>	***
Benzyl alcohol	1865	A	110 <sup>a</sup>	135 <sup>b</sup>	260 <sup>c</sup>	**
2-Phenyl ethanol	1902	A	13638 <sup>a</sup>	14338 <sup>a</sup>	19085 <sup>b</sup>	**
<b>Total of higher alcohols</b>			<b>113,556</b>	<b>137,539</b>	<b>162,805</b>	
<b>Esters</b>						
Isoamyl acetate	1125	A	1809 <sup>a</sup>	6641 <sup>b</sup>	5221 <sup>c</sup>	***
Ethyl hexanoate	1230	A	510 <sup>a</sup>	758 <sup>b</sup>	855 <sup>c</sup>	***
Hexyl acetate	1307	B	22 <sup>a</sup>	65 <sup>b</sup>	83 <sup>c</sup>	***
Ethyl lactate	1353	A	1545 <sup>a</sup>	1625 <sup>b</sup>	5841 <sup>c</sup>	*
Etyhl octanoate	1430	A	383 <sup>a</sup>	800 <sup>b</sup>	631 <sup>c</sup>	***
Ethyl decanoate	1635	A	283 <sup>a</sup>	657 <sup>b</sup>	780 <sup>c</sup>	***
2-Phenyl ethyl acetate	1820	B	209 <sup>a</sup>	394 <sup>b</sup>	780 <sup>c</sup>	***
Ethyl dodecanoate	-	C	112 <sup>a</sup>	369 <sup>b</sup>	279 <sup>c</sup>	***
Ethyl phenyl lactate	-	C	13 <sup>a</sup>	34 <sup>b</sup>	78 <sup>c</sup>	***
<b>Total of esters</b>			<b>4886</b>	<b>11,343</b>	<b>14,548</b>	
<b>Volatile Acids</b>						
Propanoic acid	-	C	56 <sup>a</sup>	72 <sup>b</sup>	71 <sup>c</sup>	*
Butyric acid	1610	B	1504 <sup>a</sup>	1190 <sup>b</sup>	1496 <sup>a</sup>	***
Hexanoic acid	1838	B	1721 <sup>a</sup>	2860 <sup>b</sup>	3665 <sup>c</sup>	***
Octanoic acid	2060	B	2888 <sup>a</sup>	5374 <sup>b</sup>	4926 <sup>c</sup>	**
Nonanoic acid	-	C	67 <sup>a</sup>	80 <sup>b</sup>	89 <sup>c</sup>	*
Decanoic acid	2357	B	899 <sup>a</sup>	2037 <sup>b</sup>	1537 <sup>c</sup>	***
Dodecanoic acid	2499	B	177 <sup>a</sup>	454 <sup>b</sup>	302 <sup>ab</sup>	***
Tetradecanoic acid	2692	B	101 <sup>a</sup>	154 <sup>b</sup>	190 <sup>c</sup>	***
Octadecanoic acid	-	C	82 <sup>a</sup>	393 <sup>b</sup>	836 <sup>c</sup>	***
<b>Total of volatile acids</b>			<b>7495</b>	<b>12,614</b>	<b>13,112</b>	
<b>Phenols</b>						
4-vinyl guaiacol	2177	A	22 <sup>a</sup>	32 <sup>b</sup>	29 <sup>c</sup>	**
4-vinyl phenol	2378	A	45 <sup>a</sup>	11 <sup>b</sup>	6 <sup>c</sup>	***
<b>Total of phenols</b>			<b>67</b>	<b>43</b>	<b>35</b>	
<b>Carbonyl Compounds</b>						
Acetoin	1312	B	405 <sup>a</sup>	816 <sup>b</sup>	802 <sup>b</sup>	***
<b>Total of carbonyl compounds</b>			<b>405</b>	<b>816</b>	<b>802</b>	
<b>TOTAL</b>			<b>126,409</b>	<b>162,355</b>	<b>191,302</b>	

<sup>1</sup> RI: Linear retention index. Iden.: 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. S: Spontaneously fermented Wine, I: Wine produced by indigenous *S. cerevisiae*, C: Wine produced by commercial *S. cerevisiae*. Sig.: Significance at which means differ in the same row as shown by analysis of variance: \*, \*\*, \*\*\* denotes significance at  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$  respectively. The SD values in the quantification of peaks did not exceed  $\pm 10\%$ .

5. Antonelli A., Castellari, L., Zambonelli, C. and Carnacini, A. *Journal of Agricultural and Food Chemistry*, 1999, **47**, 1139.
6. Baker, C.D., *Recommended Methods of Analysis*, Institute of Brewing, London, 1991.
7. Barnett, C.D., Payne, R.W. and Yarrow, D., *Yeasts: Characteristics and Identification*, CUP, Cambridge, 1983.
8. Baumes, R., Cordonnier, R., Nitz, S. and Drawert, F., *Journal of the Science of Food and Agriculture*, 1986, **37**, 927.
9. Blanch, G.P., Peglero, G., Herraiz, M. and Taberea T.A., *Journal of Chromatography Science*, 1991, **29**, 11.
10. Boulton, R.B., Singleton, V.L., Bisson L.F. and Kunkee, R.E., *Principles and Practices of Winemaking*, Chapman and Hall, New York, 1996.
11. Cabaroğlu, T., Canbaş, A., Baumes, R., Bayonove C., Lepoutre C.P. and Günata Z. *Journal of Food Science*, 1997, **62**, 680.
12. Canbas, A., Unal, U., Deryaoglu, A., Erten, H. and Cabaroğlu, T., *Gıda*, 1995, **20**, 281.
13. Degre, R., In *Wine Microbiology and Biotechnology*, ed. G.M. Fleet, Harwood Academic Publishers, Chur, Switzerland, 1993, 421.
14. Deryaoglu, A., Colin, J.L. and Canbas, A., *Gıda*, 1997, **20**, 281.
15. Erten, H., *The Production of Low Alcohol Wines by Aerobic Yeasts*, PhD Thesis, Heriot-Watt University, Scotland, 1997.
16. Etiévant, P.X., In *Volatile Compounds. In Food and Beverages*, ed. H. Maarse, Marcel Dekker, New York, 1991, 483.
17. Fleet, G.H. and Heard, G.M., In *Wine Microbiology and Biotechnology*, ed. G.M. Fleet, Harwood Academic Publishers, Chur, Switzerland, 1993, 27.
18. Fidan Y. and Yavaş I., *Türkiye 1. Ulusal Bahçe Bitkileri Kongresi Cilt II (Sebze-Bağ-Süs Bitkileri)*, Ege Üniversitesi Ziraat Fakültesi, 13-16 Ekim 1992, İzmir, 1992, 523.
19. Gill, J.V., Mateo, J.J., Jimenez, M., Pastor, A. and Huerta, T., *Journal of Food Science*, 1996, **61**, 1247.
20. Giudici, P., Zambonelli, C. and Kunkee, R.E., *American Journal of Enology and Viticulture*, 1993, **44**, 17.
21. Herraiz, T., Reglero, G., Herraiz, M., Martin-Alvarez, P.J. and Cabezudo, M.D., *American Journal of Enology and Viticulture*, 1990, **41**, 313.
22. Lehtonen, M. and Jounela-Eriksson, P., In *Flavour of Distilled Beverages: Origin and Development*, ed. J.R. Piggott, Ellis Harwood, London, 1983, 64.
23. López, R., Ferreira, V., Hernández, P. and Cacho, J.F., *Journal of The Science of Food and Agriculture*, 1999, **79**, 1461.
24. Mateo, J.J., Jimenez M., Huerta T. and Pastor A., *International Journal of Food Microbiology*, 1991, **14**, 153.
25. Mauricio, J.C., Moreno, J., Zea, I., Ortega, J.M. and Medina, M., *Journal of the Science of Food and Agriculture*, 1997, **75**, 155.
26. Nykänen, L. and Suomalainen, H., *Aroma of Beer, Wine and Distilled Alcoholic Beverages*, D. Reidel Publishing Com. Dordrecht, Holland, 1983.
27. Ough, C.S. and Amerine, M.A., *Methods for Analyses of Musts and Wines*, 2nd. edn. John Wiley and Sons, New York, 1988.
28. Rankine, B.C. *Journal of the Science of Food and Agriculture*, 1967, **18**, 584.
29. Rapp, A. and Versini, G., In *Proceedings of International Symposium on Nitrogen in Grapes and Wine*, ed. J.M. Rantz, The American Society of Enology and Viticulture, Davis, CA, 1991, 156.
30. Reed, G. and Nagodawithana, T.W., *American Journal of Enology and Viticulture*, 1988, **39**, 83.
31. Regodón, J.A., Pérez, F., Valdés, M.E., De Miguel, C. and Ramírez, M., *Food Microbiology*, 1997, **14**, 247.
32. Ribereau-Gayon, P., In *Flavour of Foods and Beverages: Chemistry and Technology*, eds. G. Charalambous, G.E. Inglett, Academic Press, London, 1978, 355.
33. Sahin, I., *Mayaların Sarap Bilesim ve Kalitesine Etkileri Uzerinde Arastirmalar*, Ankara Universitesi, Ziraat Fakultesi yayinlari, 821, Ankara, 1982.
34. Schneider, R., Baumes, R., Bayonove, C. and Razungles, A., *Journal of Agricultural and Food Chemistry*, 1988, **46**, 3230.
35. Simpson, R.F., *Food Technology in Australia*, 1979, **31**, 516.
36. Soles, R.M., Ough, C.S. and Kunkee, R.E., *American Journal of Enology and Viticulture*, 1982, **33**, 94.
37. Ubeda-Iranzo, J.F., González-Magana, F. and Gonzales-Viñas, M.A., *Food Control*, 2000, **1**, 143.

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